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(54) **Title:** DIPHENYL ETHER DERIVATIVES AND THEIR USES AS HEPARANASE INHIBITORS

(57) **Abstract:** The invention provides diphenyl ether compounds as heparanase inhibitors suitable for treatment of diseases and disorders caused by or associated with heparanase catalytic activity such as cancer, inflammatory disorders and autoimmune diseases.

DIPHENYL ETHER DERIVATIVES AND THEIR USES AS HEPARANASE INHIBITORS

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to heparanase inhibitors, particularly to certain diphenyl ether derivatives, and to their use in the treatment of diseases and disorders caused by or associated with heparanase catalytic activity such as cancer, inflammatory disorders and autoimmune diseases.

Heparan sulfate proteoglycans (HSPGs) are ubiquitous macromolecules associated with the cell surface and with the extracellular matrix (ECM) of various tissues. They consist of a protein core to which several linear heparan sulfate (HS) chains are covalently attached. Studies on the involvement of ECM molecules in cell attachment, growth and differentiation revealed a central role of HSPGs in embryonic morphogenesis, angiogenesis, neurite outgrowth, tissue repair, and metastasis. HSPGs are also prominent components of blood vessels. In capillaries they are found mainly in the subendothelial basement membrane, where they support proliferating and migrating endothelial cells and stabilize the structure of the capillary wall.

Several cellular enzymes such as collagenase IV, plasminogen activator, cathepsin B, and elastase are thought to be involved in the degradation of basement membrane. Another enzyme of this type is heparanase, an endo-β-D-glucuronidase that cleaves HS at specific intrachain sites (Nakajima et al., 1984). Heparanase released from cells removes HS molecules from the basement membrane resulting in increase of basement membrane permeability. Heparanase also facilitates proteolytic degradation of the core structural components such as type IV collagen in collaboration with gelatinases. Thus, blood-borne cells accomplish penetration through the basement membrane. In fact, HS catabolism is observed in wound repair, inflammation, and in diabetes.

Expression of heparanase was found to correlate with the metastatic potential of mouse lymphoma (Vlodavsky et al., 1983), fibrosarcoma and melanoma cells (Nakajima et al., 1988). Similar correlation was observed in human breast, colon, bladder, prostate, and liver carcinomas (Vlodavsky et al., 1999). Moreover, elevated levels of heparanase were detected in sera of metastatic tumor bearing animals (Nakajima et al., 1988) and of

cancer patients, in urine of highly metastatic patients (Vlodavsky et al., 1997), and in tumor biopsies (Vlodavsky et al., 1988). Treatment of experimental animals with heparanase substrates or inhibitors (e.g., non-anticoagulant species of low molecular weight heparin and polysulfated saccharides) considerably reduced the incidence of lung metastases induced by B16-F10 melanoma, pancreatic adenocarcinoma, Lewis lung carcinoma, and mammary adenocarcinoma cells (Vlodavsky et al., 1994; Nakajima et al., 1988; Parish et al., 1987; Lapierre et al., 1996), indicating that heparanase inhibitors may inhibit tumor cell invasion and metastasis.

Heparanase is involved also in primary tumor angiogenesis. Most primary solid tumors (1-2 mm diameter) obtain their oxygen and nutrient supply through a passive diffusion from pre-existing blood vessels, however the increase in their mass beyond this size requires angiogenesis. Heparin-binding polypeptides such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are highly mitogenic for vascular endothelial cells, and are among the most potent inducers of angiogenesis. bFGF has been extracted from the subendothelial ECM produced in vitro, and from basement membranes of cornea, suggesting that ECM may serve as a reservoir for bFGF. Immunohistochemical staining revealed the localization of bFGF in basement membranes of diverse tissues and blood vessels. bFGF binds to HSPG in the ECM and can be released in an active form by HS-degrading enzymes. Heparanase expressed by platelets, mast cells, neutrophils, and lymphoma cells was found to be involved in the release of active bFGF from ECM and basement membranes, suggesting that heparanase activity may not only function in cell migration and invasion, but may also elicit an indirect neovascular response (Elkin et al., 2001).

Heparanase catalytic activity correlates with the ability of activated cells of the immune system to leave the circulation and elicit both inflammatory and autoimmune responses. Interaction of platelets, granulocytes, T and B lymphocytes, macrophages, and mast cells with the subendothelial ECM is associated with degradation of HS by heparanase (Vlodavsky et al., 1992). The enzyme is released from intracellular compartments (e.g., lysosomes, specific granules) in response to various activation signals (e.g., thrombin, calcium ionophore, immune complexes, antigens, mitogens), suggesting its regulated involvement in inflammatory sites and in autoimmune diseases. Indeed, treatment of experimental animals with heparanase substrates (e.g., non-

anticoagulant species of low molecular weight heparin) markedly reduced the incidence of experimental autoimmune encephalomyelitis (EAE), adjuvant arthritis and graft rejection, indicating that heparanase inhibitors may inhibit autoimmune and inflammatory diseases (Lider et al., 1989).

Heparanase inhibitors have been proposed for treatment of human metastasis, for example, derivatives of siastatin B (Nishimura et al., 1994; Kawase et al., 1995), a pyran derivative isolated from the fungal strain *Acremonium* sp. MT70646 (PCT/KR00/01493), suramin, a polysulfonated naphthylurea (Nakajima et al., 1991), sulfated oligosaccharides, e.g., sulfated maltotetraose and maltohexaose (Parish et al., 1999), and sulfated polysaccharides (Parish et al., 1987; Lapierre et al., 1996).

U.S. Patent No. 5,968,822 discloses a polynucleotide encoding a polypeptide having heparanase catalytic activity and host cells, particularly insect cells, expressing said polypeptide. The recombinant polypeptide having heparanase activity is said to be useful for potential treatment of several diseases and disorders such as wound healing, angiogenesis, restenosis, inflammation and neurodegenerative diseases as well as for development of new drugs that inhibit tumor cell metastasis, inflammation and autoimmunity. International Patent Publication No. WO 99/57244 of the present applicants discloses bacterial, yeast and animal cells and methods for overexpressing recombinant heparanase in cellular systems. U.S. Patent No. 6,190,875, assigned to the present applicants, discloses methods of screening agents inhibiting heparanase catalytic activity and hence potentially inhibiting tumor metastasis, autoimmune and inflammatory diseases which comprises interacting a native or recombinant heparanase enzyme with a heparin substrate in the presence or absence of an agent and determining the inhibitory effect of said agent on the catalytic activity of said heparanase enzyme towards said heparin substrate. Both U.S. 5,968,822 and U.S. 6,190,875 and further WO 99/57244 are herein incorporated by reference in their entirety as if fully disclosed herein.

Japanese Patent Publications Nos. 06-016597, 06-016601, 05-301849 and 05-271156 disclose certain 1-alkoxy-2,6-diphenoxybenzene derivatives said to exhibit antineoplastic activity. The heparanase inhibitors of the present invention have not been disclosed nor suggested in said publications.

SUMMARY OF THE INVENTION

The present invention provides, in one aspect, a pharmaceutical composition comprising a pharmaceutically acceptable carrier and at least one heparanase inhibitor selected from a diphenyl ether derivative of the general Formula I hereinafter or a pharmaceutically acceptable salt thereof.

The pharmaceutical composition of the invention is particularly useful for the treatment of diseases and disorders caused by or associated with heparanase catalytic activity such as, but not limited to, cancer, inflammatory disorders and autoimmune diseases.

In another aspect, the present invention relates to the use of a diphenyl ether derivative of the general Formula I for the manufacture of a pharmaceutical composition. In one embodiment, said composition is for the treatment of diseases and disorders caused by or associated with heparanase catalytic activity such as cancer, inflammatory disorders and autoimmune diseases.

In a further aspect, the present invention provides a novel diphenyl ether derivative of the general Formula I.

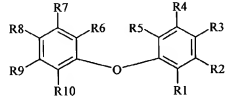
In still another aspect, the present invention relates to a method for treatment of a patient suffering from a disease or disorder caused by or associated with heparanase catalytic activity such as cancer, an inflammatory disorder or an autoimmune disease, which comprises administering to said patient an effective amount of a diphenyl ether derivative of the general Formula I.

BRIEF DESCRIPTION OF THE FIGURES

Figs. 1A-B show transmigration rates through a Matrigel filter of mock-transfected (lacking heparanase) Eb murine lymphoma cells (Eb-cells) and *hepa*-transfected Eb murine lymphoma cells (Eb-heparanase cells) overexpressing heparanase, in the absence (-) or in the presence (+) of the chemoattractant SDF-1 (Fig. 1A), and of *hepa*-transfected Eb murine lymphoma cells (Eb-heparanase cells) overexpressing heparanase untreated (control) or treated with the compound herein identified as **Compound 1** (Fig. 1B).

DETAILED DESCRIPTION OF THE INVENTION

According to the present invention, pharmaceutical compositions are provided for treatment of diseases and disorders caused by or associated with heparanase catalytic activity, said compositions comprising a pharmaceutically acceptable carrier and at least one heparanase inhibitor which is a diphenyl ether compound of the general Formula I:



(I)

wherein

R1, R5, R6 and R7 each independently represents hydrogen or halogen;

R2, R3, R4 and R8 each independently represents hydrogen, halogen, nitro, -OR', -SR', -NR11R12, -COOR', -CONR11R12, -SO_3H, -SO_2NR11R12, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, C6-C14 aryl or heteroaryl;

R9 and R10 each independently is hydrogen or halogen, or R9 and R10 together with the carbon atoms to which they are attached form a condensed benzene ring;

R11 and R12 each independently represents hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or heteroaryl;

or R11 is H and R12 is C2-C7 alkanoyl or C7-C15 aroyl, or R11 and R12 together with the N atom to which they are attached form a saturated 5-7 membered heterocyclic ring containing one to three heteroatoms selected from N, O and/or S;

R' is hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or hetcroaryl;

"heteroaryl" in radicals R2, R3, R4, R8, R11, R12 and R' is a radical derived from a mono- or poly-cyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

any "C1-C6 alkyl", "C2-C7" alkanoyl and "C2-C6" alkenyl in radicals R2, R3, R4, R8 R11, R12 and R' may be substituted by at least one group selected from halogen, -OR', -SR',-NR11R12, -COOR', -CONR11R12, nitro, -SO_3H, -SO_2NR11R12, C6-C14 aryl, and heteroaryl;

any "C6-C14 aryl", "C7-C15 aroyl" and "heteroaryl" in radicals R2, R3, R4, R8 R11, R12 and R' may be substituted by at least one group selected from halogen, -OR', -

SR', -NR11R12, -COOR', -CONR11R12, nitro, -SO_3H, -$SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, and C5-C6 cycloalkyl;

and pharmaceutically acceptable salts thereof.

As used herein the term "C1-C6 alkyl" typically refers to a straight or branched alkyl radical having 1-6 carbon atoms and includes for example methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert-butyl, n-heptyl, 2,2-dimethylpropyl, n-hexyl and the like. The term "C2-C6 alkenyl" refers to straight or branched hydrocarbon radicals having 2-6 carbon atoms and one double bond, preferably a terminal double bond, and includes for example vinyl, prop-2-en-1-yl, but-3-en-1-yl, pent-4-en-1-yl, and hex-5-en-1-yl. The term "C1-C6 alkoxy" refers to the group C1-C6 alkyl-O-, wherein C1-C6 alkyl is as defined above. Examples of alkoxy are methoxy, ethoxy, hexoxy and the like. The term "C2-C7 alkanoyl" refers to the group C1-C6 alkyl-CO-, wherein C1-C6 alkyl is as defined above. Examples of alkanoyl are acetyl, propanoyl, butanoyl, and hexanoyl.

The term "C6-C14 aryl" refers to an aromatic carbocyclic group having 6 to 14 carbon atoms consisting of a single ring or multiple condensed rings such as phenyl, naphthyl, and phenanthryl optionally substituted as defined above. The term "C7-C15 aroyl" refers to the group C6-C14 aryl-CO-, wherein C6-C14 aryl is as defined above. Particular examples are benzoyl, naphthoyl, phenanthroyl and anthroyl.

The term "heteroaryl" refers to a radical derived from a mono- or poly-cyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S. Particular examples are pyridyl, pyrrolyl, furyl, thienyl, imidazolyl, oxazolyl, quinolinyl, thiazolyl, pyrazolyl, 1,3,4-triazinyl, 1,2,3-triazinyl, benzofuryl, isobenzofuryl, indolyl, imidazo[1,2-a]pyridyl, benzimidazolyl, benzthiazolyl and benzoxazolyl. It is to be understood that when a polycyclic heteroaromatic ring is substituted, the substitutions may be in any of the carbocyclic and/or heterocyclic rings.

The term "halogen" refers to fluoro, chloro, bromo or iodo.

The group –$NR11R12$ may be –NH_2, when R11 and R12 are both hydrogen, or R11 is hydrogen and R12 is C2-C7 alkanoyl or C7-C15 aroyl, as defined above, or R11 and R12 together with the nitrogen atom to which they are attached form a saturated 5-7 membered heterocyclic ring, preferably a 6-membered ring, optionally containing at least one further heteroatom selected from nitrogen, oxygen and/or sulfur. Such rings may be substituted, for example with one or two C1-C6 alkyl groups, preferably at the further N

atom. Examples of such rings include, without being limited to, pyrrolidino, piperidino, morpholino, thiomorpholino, benzodiazepino, piperazino, N-C1-C6 alkylpiperazino, e.g. N-methylpiperazino and the like.

Also contemplated by the present invention are pharmaceutically acceptable salts of the compounds of Formula I, both salts formed by any carboxy or sulfo groups present in the molecule and a base as well as acid addition and/or base salts.

Pharmaceutically acceptable salts are formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Examples of metals used as cations are sodium, potassium, magnesium, calcium, and the like. Examples of suitable amines are N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, N-methylglucamine, and procaine (see, for example, Berge S. M., et al., "Pharmaceutical Salts," (1977) J. of Pharmaceutical Science, 66:1-19). The salts can also be pharmaceutically acceptable quaternary salts such as a quaternary salt of the formula – NRR'R" + Z' wherein R, R' and R" each is independently hydrogen, alkyl or benzyl and Z is a counterion, including chloride, bromide, iodide, O-alkyl, toluenesulfonate, methylsulfonate, sulfonate, phosphate, or carboxylate.

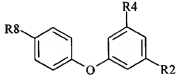
Pharmaceutically acceptable acid addition salts of the compounds include salts derived from inorganic acids such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydriodic, phosphorous, and the like, as well as salts derived from organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, alkanedioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, etc. Such salts thus include sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, nitrate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, caprylate, isobutyrate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, mandelate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, phthalate, benzenesulfonate, toluenesulfonate, phenylacetate, citrate, lactate, maleate, tartrate, methanesulfonate, and the like. Also contemplated are salts of amino acids such as arginate and the like and gluconate or galacturonate (see, for example, Berge S. M., et al., "Pharmaceutical Salts," (1977) J. of Pharmaceutical Science, 66:1-19).

The acid addition salts of said basic compounds are prepared by contacting the free base form with a sufficient amount of the desired acid to produce the salt in the

conventional manner. The free base form may be regenerated by contacting the salt form with a base and isolating the free base in the conventional manner. The free base forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free base for purposes of the present invention.

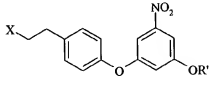
The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in the conventional manner. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present invention.

In a preferred embodiment of the present invention, the pharmaceutical composition comprises a compound of the formula Ia:



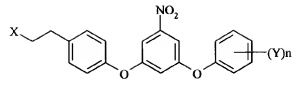
(Ia)

wherein R2 is –OR', R4 is nitro, and R8 is C1-C6 alkyl optionally substituted by at least one group selected from halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C6-C14 aryl, and heteroaryl, preferably by an ethyl substituted at the terminal carbon by X, as depicted in formula Ib:



(Ib)

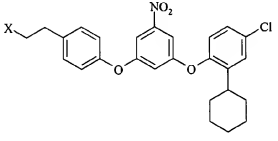
wherein X may be -COOR' or -CONR11R12, wherein R', R11 and R12 are as defined hereinabove. According to this embodiment, R' may be a phenyl substituted by at least one group Y, as depicted in formula Ic:



(Ic)

wherein Y is halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2$ NR11R12, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl or C5-C6 cycloalkyl; n is an integer from 0 to 5, X is –COOR' or –CONR11R12, and R', R11 and 12 are as defined above.

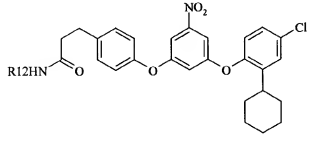
In a preferred embodiment, in the compound of formula Ic, n is 2 and one Y is halogen, preferably Cl, at the para position to the oxygen, and another Y is C6 cycloalkyl at the ortho position to the oxygen, as exemplified in formula Id:



(Id)

In the compound of formula Id, when radical X is -COOR' and R' is hydrogen, there is obtained the compound herein designated **Compound 1** in the Appendix A just before the Claims. This compound is described in the literature [CAS No. 332406-48-1] but no biological activity is disclosed for it.

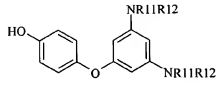
In another embodiment of the present invention, in the compound of formula Id, X is –CONR11R12, and R11 is preferably hydrogen as depicted in the formula Ie:



(Ie)

According to this embodiment, when R12 is ethyl substituted at the terminal position by $-SO_3H$, there is obtained the novel compound herein designated **Compound 2** in the Appendix A just before the Claims.

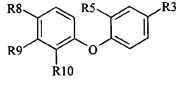
In yet another embodiment of the present invention, in the compound of formula Ia, R2 and R4 are NR11R12 and R8 is –OH as depicted in formula If:



(If)

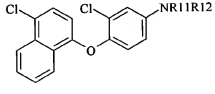
In the compound of formula If, R11 may be hydrogen and R12 may be acetyl substituted at the alpha-position by 2-methyl-phenoxy, as exemplified by the compound herein designated **Compound 3** in the Appendix A just before the Claims. This compound is described in the literature [CAS No. 313249-03-5] but no biological activity is disclosed for it.

In another preferred embodiment, the composition comprises a compound of the formula Ig:



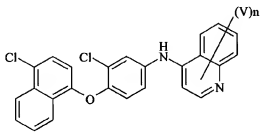
(Ig)

wherein R3 is preferably -NR11R12, R5 and R8 are halogen, preferably Cl, and R9 and R10 together with the carbon atoms to which they are attached form a condensed benzene ring, as is depicted in formula Ih:



(Ih)

According to this embodiment, in the compound of formula Ih, R11 may be hydrogen and R12 may be heteroaryl, preferably quinolinyl, substituted by 1 to 6 V groups as depicted in formula Ii:



(Ii)

In one preferred embodiment, n is 2 and one V is alkyl, preferably methyl, and another V is alkoxy, preferably methoxy, as exemplified by the compound herein designated **Compound 4** in the Appendix A just before the Claims. This compound is described in the literature [CAS No. 301354-99-4] but no biological activity is disclosed for it.

The present invention further encompasses the novel **Compound 2**.

Compounds 1, **3** and **4** are prepared in multi-step syntheses according to the procedures of Eastmond et al.1998, and Shevelev et al, 1995, as shown in **Scheme 1**. Thus, an appropriate nitrobenzene derivative, such as trinitrobenzene, dinitrobenzene, or 1-chloro-4-nitronaphthalene is reacted with an appropriately substituted phenol, such as p-chlorophenol, or p-dihydroxybenzene, in the presence of a strong base such as KOH or lithium hydroxide (LiOH). The product, a substituted diphenyl ether, may then be optionally further derivatized by a) further ipso-attack on the remaining nitro groups, b) by reduction of the remaining nitro groups and acylation of the resulting amino groups, or c) by further manipulation on the various other functional groups that may be present.

Thus, **Compound 1** is prepared in several steps, as shown in **Scheme 2.** Friedel-Crafts alkylation of p-chlorophenol by cyclohexene in the presence of $AlCl_3$, gave in step (a) a disubstituted phenol, ***intermediate i,*** which was then reacted at room temperature with trinitrobenzne in the presence of lithium hydroxide, as shown in step b, thus obtaining ***intermediate ii.*** Further, ***intermediate ii*** was reacted with 3-(4-

hydroxyphenyl)propionic acid, under identical conditions to those employed in step b, thus affording the desired **Compound 1**.

Compound 2 was prepared by treating **Compound 1** with taurine (2-aminoethane sulfonic acid) in the presence of a coupling agent such as EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline), in ethanol, as is shown in **Scheme 2**.

Although the procedures given are used specifically for the synthesis of the diphenyl ether derivatives of this invention, the methods apply widely to analogous compounds of Formula I, given appropriate consideration to protection and deprotection of reactive functional groups by methods standard to the art of Organic Chemistry. For example, in order to prevent unwanted side reactions, hydroxy groups generally need to be converted to ethers or esters during chemical reactions at other sites in the molecule. The hydroxy protecting group is readily removed to provide the free hydroxy group. Amino groups and carboxylic acid groups are similarly derivatized to protect then against unwanted side reactions. Typical protecting groups, and methods for attaching and cleaving them, are described fully by Greene and Wuts in Protective Groups in Organic Synthesis, John Wiley and Sons, New-York (2nd Ed, 1991) and McOmie, Protective Groups in Organic Chemistry, Plenum Press, New-York, 1973.

The inhibitory effect of the compounds of the present invention on heparanase activity can be evaluated by several methods carried out in vitro, ex vivo, or in vivo.

Some of the in vitro assays used according to the present invention were described in US 6,190,875. In these assays, heparanase is incubated with a heparanase substrate in the presence and in the absence of a compound of the present invention, and the inhibitory effect of the compound on the catalytic activity of the heparanase on its substrate is evaluated.

The heparanase may be natural mammalian heparanase, such as human heparanase purified as described in U.S. Patent 5,362,641 or, preferably, recombinant mammalian, e.g. human or mouse recombinant heparanase as described in US 5,968,822, US 6,190,875, and WO 99/57244, in purified or non-purified form. A source of non-purified recombinant heparanase is, for example, an extract of cells in which mammalian heparanase cDNA is expressed.

The heparanase substrate may be a natural heparan sulfate substrate, or an alternative substrate of the enzyme as described in U.S. 6,190,875, for example, heparin

(e.g. heparin immobilized on a gel such as Sepharose), heparin fragments (e.g. several species of low molecular weight heparin), modified non-anticoagulant species of heparin, other sulfated polysaccharides (e.g. pentosan polysulfate), soluble HSPG or ECM.

Evaluation of the inhibitory effect can be carried out, for example, as described in US 6,190,875, by a size separation assay adapted for detection of degradation products of the heparanase substrate. Examples of such assays include gel electrophoresis and column chromatography.

Qualitative and quantitative evaluation of the catalytic activity of heparanase on its substrate and the inhibitory effect of a candidate inhibitor can be effected, for example, by colorimetric assays. Any colorimetric assay based on any color producing reaction is envisaged by the invention, be it a simple color reaction, which is readily detectable, or a fluorimetric or a luminiscent (e.g., chemiluminiscent) reaction, which are readily detectable by fluorescence detecting techniques. Examples of such suitable colorimetric assays include, but are not limited to, the dimethylmethylene blue (DMB), tetrazolium blue and carbazole assays. Qualitative colorimetric assays include the dimethylmethylene blue (DMB) assay, which yields color shift in the presence of polyanionic compounds such as sulfated glycosaminoglycans having different sizes that are released from the substrate (soluble or immobilized), and the carbazole assay, which detects uronic acid derivatives present in complete hydrolyzates of products released from an immobilized substrate, both assays being applicable for crude extracts of heparanase and for the purified enzyme as well.

In a preferred embodiment, a quantitative evaluation is desired and the preferred in vitro assays are those which are adapted for detection of reducing moieties associated with degradation products of the heparanase substrate, preferably a reducing sugar assay. An example of a quantitative colorimetric assay is the tetrazolium blue assay which allows colorimetric detection of reducing moieties released from the substrate, e.g. heparan sulfate, which may be present either in soluble or immobilized form.

Another possibility, although less preferred, consists in evaluating the catalytic activity of heparanase on the substrate by radioactive techniques, in which case the substrate used is radiolabeled, either in vitro or metabolically.

The ex vivo assays for evaluating the inhibitory effect of the compounds on heparanase activity include angiogenic sprout formation and transmigration assays. The

angiogenic sprout formation assay is carried out in the rat aorta model (Nicosia et al., 1997; Nicosia and Ottinetti, 1990), whereby rat aorta rings are embedded in a basement membrane-like matrix composed of ECM-derived proteins such as laminin and collagen type IV, and HSPG, thus constituting a relevant heparanase substrate. The rings then develop angiogenic sprouts and angiogenesis can be quantitated. The compounds to be tested are added to the embedded aortic rings and their effect on angiogenic sprout formation is then evaluated.

In the ex vivo transwell migration assay, immune cell migration is evaluated, optionally in the presence of a chemoattractant factor such as stromal cell-derived factor 1 (SDF-1), a process which mimics in vivo extravasation of immune cells from the vasculature to sites of inflammation. In this assay, immune cells such as lymphocytes are let to migrate from the upper to the lower chamber through a transwell filter coated with a basement membrane-like matrix composed of ECM-derived proteins. The migration rate of the cells through the filter is then evaluated by counting the number of cells migrated through the filter (e.g. using a FACSort) compared to the number of cells added on top of the upper chamber. Over expression of heparanase in the immune cells results in an increase in the transmigration rate of the cells while addition of a heparanase inhibitor reduces the transmigration rate of the cells.

The inhibitory effect of the compounds on heparanase activity may be also assayed in vivo, for example, using the primary tumor growth or metastasis animal models or the sponge inflammation assay.

In the primary tumor animal model, animals are injected subcutaneously (s.c.) with tumor cells and treated with the heparanase inhibitors. Tumor growth is measured when animals in untreated control group start to die. For example, primary tumors may be generated with B16-F1 melanoma cells or with a highly metastatic subclone thereof injected s.c. into the flanks of mice. The mice are treated with heparanase inhibitors injected intraperitoneally (i.p.) twice a day starting 4 days after cell injection and are sacrificed and the tumor measured about 3 weeks after cell injection.

In the metastasis animal model, animals are injected intravenously (i.v.) with tumor cells and treated with the heparanase inhibitors. The number of lung metastasis is counted when animals in untreated control group start to die or about 3 weeks after cell injection. For example, metastasis may be generated with B16-F1 melanoma cells or with

a highly metastatic subclone thereof injected i.v. to mice. The mice are treated with heparanase inhibitors injected i.p. at certain times following cell injection, and are then sacrificed and the number of lung metastasis is counted.

In the sponge inflammation assay, polyvinyl alcohol (PVA) sponges are implanted under the mouse skin and the mouse is kept untreated or is treated with a test inhibitor agent. One day later, the mouse is sacrificed, the sponges are taken out, squeezed into a tube and the number of cells in each sample is determined. After centrifugation, the myeloperoxidase (MPO) content may be determined in a suspension of the cell pellets, and the TNF-α content in the supernatant of the sample. This assay mimics the inflammatory reaction resulting from the presence of a foreign body in the organism.

The heparanase inhibitors of the present invention can be used for the treatment of diseases and disorders caused by or associated with heparanase catalytic activity such as, but not limited to, cancer, inflammatory disorders and autoimmune diseases.

Thus, in one embodiment of the present invention, the compounds can be used for inhibition of angiogenesis, and are thus useful for the treatment of diseases and disorders associated with angiogenesis or neovascularization such as, but not limited to, tumor angiogenesis, ophthalmologic disorders such as diabetic retinipathy and macular degeneration, particularly age-related macular degeneration, reperfusion of gastric ulcer, and also for contraception or for inducing abortion at early stages of pregnancy.

In another embodiment of the invention, the compounds of general formula I are useful for treatment or inhibition of a malignant cell proliferative disease or disorder.

According to this embodiment and due to the angiogenesis inhibitory activity of the compounds, they can be used for the treatment or inhibition of non-solid cancers, e.g hematopoictic malignancies such as all types of leukemia, e.g. acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), myelodysplastic syndrome (MDS), mast cell leukemia, hairy cell leukemia, Hodgkin's disease, non-Hodgkin's lymphomas, Burkitt's lymphoma and multiple myeloma, as well as for the treatment or inhibition of solid tumors such as tumors in lip and oral cavity, pharynx, larynx, paranasal sinuses, major salivary glands, thyroid gland, esophagus, stomach, small intestine, colon, colorectum, anal canal, liver, gallbladder, extrahepatic bile ducts, ampulla of vater, exocrine pancreas, lung, pleural mesothelioma, bone, soft tissue sarcoma, carcinoma and

malignant melanoma of the skin, breast, vulva, vagina, cervix uteri, corpus uteri, ovary, fallopian tube, gestational trophoblastic tumors, penis, prostate, testis, kidney, renal pelvis, ureter, urinary bladder, urethra, carcinoma of the eyelid, carcinoma of the conjunctiva, malignant melanoma of the conjunctiva, malignant melanoma of the uvea, retinoblastoma, carcinoma of the lacrimal gland, sarcoma of the orbit, brain, spinal cord, vascular system, hemangiosarcoma and Kaposi's sarcoma.

It is to be understood that the compounds of the general formula I are useful for treating or inhibiting tumors at all stages, namely tumor formation, primary tumors, tumor progression or tumor metastasis.

The compounds of general formula I are also useful for inhibiting or treating cell proliferative diseases or disorders such as psoriasis, hypertrophic scars, acne and sclerosis/scleroderma, and for inhibiting or treatment of other diseases or disorders such as polyps, multiple exostosis, hereditary exostosis, retrolental fibroplasia, hemangioma, and arteriovenous malformation.

In a further embodiment, the compounds of general formula I are useful for treatment of or amelioration of inflammatory symptoms in any disease, condition or disorder where immune and/or inflammation suppression is beneficial such as, but not limited to, treatment of or amelioration of inflammatory symptoms in the joints, musculoskeletal and connective tissue disorders, or of inflammatory symptoms associated with hypersensitivity, allergic reactions, asthma, atherosclerosis, otitis and other otorhinolaryngological diseases, dermatitis and other skin diseases, posterior and anterior uveitis, conjunctivitis, optic neuritis, scleritis and other immune and/or inflammatory ophthalmic diseases.

In another preferred embodiment, the compounds of formula I are useful for treatment of or amelioration of an autoimmune disease such as, but not limited to, Eaton-Lambert syndrome, Goodpasture's syndrome, Grave's disease, Guillain-Barré syndrome, autoimmune hemolytic anemia (AIHA), hepatitis, insulin-dependent diabetes mellitus (IDDM), systemic lupus erythematosus (SLE), multiple sclerosis (MS), myasthenia gravis, plexus disorders e.g. acute brachial neuritis, polyglandular deficiency syndrome, primary biliary cirrhosis, rheumatoid arthritis, scleroderma, thrombocytopenia, thyroiditis e.g. Hashimoto's disease, Sjögren's syndrome, allergic purpura, psoriasis, mixed connective tissue disease, polymyositis, dermatomyositis, vasculitis, polyarteritis

nodosa, polymyalgia rheumatica, Wegener's granulomatosis, Reiter's syndrome, Behçet's syndrome, ankylosing spondylitis, pemphigus, bullous pemphigoid, dermatitis herpetiformis, Crohn's disease or autism.

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. The carrier(s) must be acceptable in the sense that it is compatible with the other ingredients of the composition and are not deleterious to the recipient thereof.

The term "carrier" refers to a diluent, adjuvant, excipient, or any other suitable vehicle. Such pharmaceutical carriers can be sterile liquids such as water and oils.

The pharmaceutical composition can be administered systemically, for example by parenteral, *e.g.* intravenous, intraperitoneal or intramuscular injection. In another example, the pharmaceutical composition can be introduced to a site by any suitable route including intravenous, subcutaneous, transcutaneous, topical, intramuscular, intraarticular, subconjunctival, or mucosal, *e.g.* oral, intranasal, or intraocular.

In one specific embodiment, the pharmaceutical composition is administered to the area in need of treatment. This may be achieved by, for example, local infusion during surgery, topical application, direct injection into the inflamed joint, directly onto the eye, etc.

For oral administration, the pharmaceutical preparation may be in liquid form, for example, solutions, syrups or suspensions, or in solid form as tablets, capsules and the like. For administration by inhalation, the compositions are conveniently delivered in the form of drops or aerosol sprays. For administration by injection, the formulations may be presented in unit dosage form, *e.g.* in ampoules or in multidose containers with an added preservative.

The compositions of the invention can also be delivered in a vesicle, in particular in liposomes. In another embodiment, the compositions can be delivered in a controlled release system.

The amount of the therapeutic or pharmaceutical composition of the invention which is effective in the treatment of a particular disease, condition or disorder will depend on the nature of the disease, condition or disorder and can be determined by standard clinical techniques. In general, the dosage ranges from about 0.01 mg/kg to

about 50-100 mg/kg. In addition, in vitro assays as well in vivo experiments may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease, condition or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems. For example, in order to obtain an effective mg/kg dose for humans based on data generated from mice or rat studies, the effective mg/kg dosage in mice or rats is divided by twelve or six, respectively.

The invention will now be illustrated by the following non-limiting examples.

EXAMPLES

For convenience and better understanding, the section of the Examples is divided into two subsections: (I) the Chemical Section describing the synthesis of the diphenyl ether compounds, and (II) the Biological Section describing the biological activity of the compounds.

I CHEMICAL SECTION

The **Compounds 1-4,** which formulas are presented in Appendix A hereinafter, are identified in the Examples by their numbers in bold. The intermediates are identified in bold italics.

Materials

All reagents were purchased from Sigma-Aldrich Israel, Ltd., (Rehovot, Israel) and were used without further purification unless stated otherwise.

Compounds 1, 3 and **4** were purchased from ChemDiv, Chemical Diversity (San Diego, CA, USA).

Example 1. General approach for the synthesis of diphenyl ether derivatives Compounds 1, 3 and 4.

Compounds 1, 3 and **4** are prepared in multi-step syntheses according to the procedures of Eastmond et al.,1998, and Shevelev et al., 1998 , as shown in **Scheme 1**.

Thus, an appropriate nitrobenzene derivative, such as trinitrobenzene, dinitrobenzene, or 1-chloro-4-nitronaphthalene is reacted with an appropriately substituted phenol, such as p-chlorophenol, or p-dihydroxybenzene, in the presence of a strong base such as KOH or lithium hydroxide (LiOH). The diphenyl ether derivative is then optionally further derivatized by further ipso-attack on the remaining nitro groups, by reduction of the remaining nitro groups and acylation of the amino groups, or by further manipulation on the various other functional groups that may be present.

Example 2. Synthesis of Compound 1

The synthesis of **Compound 1** was achieved in 3 steps as shown in Scheme 2, as follows:

(step a): $AlCl_3$ (5gr, 37.3mmol) was added in one portion to a cold solution of 4-chlorophenol (5.5 g, 42.6 mmol) and cyclohexene (3 g, 42.9 mmol) in dry chlorobenzene (100 mL). The reaction mixture was allowed to warm to room temperature and was stirred for 8 hours. Cold water (200 ml) was then added to the reaction mixture and the organic phase was separate and dried over $MgSO_4$. The solvent was evaporated under reduced pressure, thus obtaining the crude solid of ***intermediate i.***

(step b): Solid LiOH (1 g, 41.7 mmol) was added to a cold solution of trinitrobenzene (9 g, 42.3 mmol) and ***intermediate i*** in dry DMF (75ml) and the reaction mixture was stirred on ice bath for 3 hours. It was then allowed to warm to room temperature, the solvent was evaporated under reduced pressure, thus obtaining ***intermediate ii*** as a dark solid.

(step c): To a cold solution of ***Intermediate ii*** and (4-hydroxy-phenyl) propionic acid (6.5 g, 42.8 mmol) in DMF (75ml) were added LiOH (1g, 41.7 mmol), the reaction mixture was stirred on ice bath for 3 hours. Then it was allowed to warm to room temperature, at which point the solvent was stripped off under reduced pressure and the solid thus obtained was dissolved in toluene (100 ml) and washed with ice water (3 x 50mL). The organic layer was dried over $MgSO_4$ and the solvent was evaporated, thus obtaining **Compound 1**.

^{1}H-NMR (DMSO-*d6*): δ= 7.5-7.3 (bm, 6H), 7.05 (d, 2H), 6.90 (s, 2H), 3.03 (m, 2H), 2.76(M,3H), 1.84(m, 4H), 1.34 (bm, 6H).

Example 3. Synthesis of Compound 2

Compound 2 was prepared in one step by treating **Compound 1** with taurine and a coupling agent as shown in **Scheme 2**, as follows:

A solution of the coupling agent EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, 350mg, 1,4 eq) in ethanol (35 mL) and taurine (2-aminoethanesulfonic acid, 125 mg, 1 mmol) in NaOH (0.5N, 2mL, 1mmol) were added to a solution of **Compound 1** (500 mg, 1 mmol) in ethanol (70mL). The reaction mixture was stirred at 50°C for 3 days, then the solvent was removed under reduced pressure and the crude **Compound 2** was obtained. The product was purified by flash chromatography using silica gel (gradient dichloromethane: methanol starting from a 95:5 to 80:20). Then, the solvent was evaporated, thus obtaining 360 mg of a light yellow powder, which was identified as **Compound 2** (57% yield).

^{1}H-NMR (DMSO-*d6*): δ= 7.74 (br t, 2H), 7.44 (d, 1H), 7.36 (t, 1H), 7.31 (m, 2H), 7.07 (m, 3H), 7.00 (t, 1H), 2.84 (t, 2H), 2.71 (q, 1H), 2.35 (t, 2H), 1.73 (t, 2H), 1.46 (t, 4H), 1.23 (t, 1H), 1.13 (t, 4H).

II BIOLOGICAL SECTION

Materials

Heparin Sepharose CL-6B was purchased from Pharmacia (Amersham Pharmacia Biotech) Uppsala, Sweden ; 1,9-Dimethylmethylene blue (DMB), tetrazolium blue and heparan sulfate were purchased from Sigma-Aldrich (Rehovot, Israel); MCDB 131 medium was purchased from Clonetics (San Diego, CA, USA); DMEM and fetal calf serum were purchased from Gibco BRL (InVitrogen Corporation, CA, USA) ; glutamine and gentamicin were purchased from Biological Industries (Bet Haemek, Israel). Matrigel was kindly provided by Dr. H. Kleinmann, NIDR, NIH, Bethesda, MD, USA.

Methods

(a) In vitro Dimethylmethylene blue (DMB) assay for heparanase activity

Heparin Sepharose CL-6B beads were added up to the top of the wells of a multiscreen column loader (Millipore). A 96-well multiscreen plate containing 0.65 μm hydrophilic, low protein binding, Durapore membrane (Millipore) was placed, upside down, on top of the multiscreen column loader. The column loader and the multiscreen plate were held together, turned over, and the beads were uniformly transferred from the column loader to the multiscreen plate. Double-distilled water (DDW) was then added to the beads, which were allowed to swell for one minute, and then washed (three times) with DDW under vacuum. Heparin concentration was estimated to be 20 μM/well.

Human recombinant heparanase of at least 50% purity was obtained by expression in the CHO cells S1-11 subclone (generated as described for CHO clones S1PPT-4 and S1PPT-8 in WO 99/57244). Active human recombinant heparanase, purified from the CHO cell extracts by ion exchange chromatography (as described for the CHO 2TT1-8 subclone in WO 99/57244), was added (5 ng/well) to a reaction mixture containing 20 mM phosphate citrate buffer, pH 5.4, 1 mM $CaCl_2$, 1 mM NaCl, and 1 mM dithiothreitol (DTT; total volume of 100 μl). After 3-hour incubation at 37° C in a incubator on a vortex shaker, the heparanase reaction products were filtered under vacuum and collected into a 96-well polystyrene flat bottom plate (Greiner Cat. No. 655101). To each well, phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA; 75 μl/well) and DMB (32 mg of DMB were dissolved in 5 ml ethanol, diluted to 1 liter with formate buffer containing 4 g sodium formate and 4 ml formic acid; 125 μl /well) were added. Color was developed after 5 minutes, and the absorbance of the samples was determined using a spectrophotometer (CECIL CE2040) at 530 nm. The absorbance correlated to heparanase activity. As a control, heparanase was added to the heparin Sepharose swollen beads in the multiscreen plate and the heparanase reaction products were filtered immediately thereafter and the absorbance of these control samples was subtracted from all other samples.

Alternatively, instead of the partially purified human recombinant heparanase enzyme as above, crude extracts of CHO cells S1-11 subclone expressing human recombinant or crude extracts of CHO cells mhG9 clone expressing mouse recombinant

heparanase (generated with the mouse heparanase cDNA as described for CHO clones expressing human recombinant heparanase in WO 99/57244) were used. The cell extracts were centrifuged and resuspended in 20 mM phosphate citrate buffer, pH 5.4 containing 50 mM NaCl. The cells were lysed by three cycles of freezing and thawing. The cell lysates were centrifuged (10000xg for 5 min), supernatants were collected and then assayed for heparanase activity using the DMB assay.

In order to examine whether a test compound exhibits an inhibitory effect on the heparanase activity, each compound was dissolved in dimethylsulfoxide (DMSO) and added, at a concentration range of 1-30 μM, to the heparin Sepharose swollen beads in the 96-multiscreen plate. The partially purified human recombinant heparanase or the crude cell extracts expressing either human or mouse recombinant heparanase was added for a 3-hour incubation and the reaction continued as described above. Color was developed and the absorbance was measured as described above. The IC_{50} value (the concentration at which the heparanase activity was inhibited by 50%) for each compound was evaluated.

(b) <u>In vitro tetrazolium blue assay for heparanase activity</u>

Human recombinant heparanase of at least 50% purity (obtained by expression in the CHO cells S1-11 subclone as described in (a) above) was added (4 ng) to each well of a 96-well microplate and incubated in a reaction mixture containing 20 mM phosphate citrate buffer, pH 5.4, 1 mM $CaCl_2$, 1 mM NaCl, and 4 μM heparan sulfate (final volume of 100 μl). After 3 hours of incubation at 37° C in an incubator on a vortex shaker, the reaction was stopped by the addition of tetrazolium blue reagent (0.11% tetrazolium blue in 0.1 M NaOH; 100 μl/well). Color was developed by incubation of the plates at 60°C for 2 hours. For each assay, a control reaction, which did not contain the substrate (heparan sulfate), was included. Color intensity was quantitatively determined in a microplate reader (Dynatech) at 580 nm. Heparanase activity was calculated as the difference between the O.D of the sample containing the substrate, and the O.D. of the sample not containing the substrate. The background O.D. produced by the substrate was also subtracted from all the samples. The absorbance correlated to heparanase activity. The IC_{50} value (the concentration at which the heparanase activity was inhibited by 50%) for each compound was evaluated.

(c) **In vivo mouse melanoma primary tumor growth assay for heparanase activity**

Instead of using a primary tumor cell line, primary tumor was generated in C57BL mice by cells herein designated FOR cells, which were generated as follows: B16-F1 mouse melanoma cells (ATCC No. 6326) were grown in DMEM containing 10% fetal calf serum, 2 mM glutamine, and 50 μg/ml gentamicin. A subclone of the B16-F1 cell line, F1-J, produced large amounts of melanin and exhibited a highly metastasis potential. These highly metastatic F1-J cells were injected to syngeneic mice (100,000 cells, s.c.). Cells from metastases that were formed were cultured in different conditions. A clone, F1-LG, designated herein FOR, was selected by its high heparanase expression and activity using the reverse transcriptase-polymerase chain reaction (RT-PCR) and the radiolabeled ECM degradation analyses, respectively, as previously described (Vlodavsky et al., 1999; U.S. 6,190,875).

FOR cells were grown in DMEM containing 10% fetal calf serum, 2 mM glutamine, and 50 μg/ml gentamicin until they reached confluence (typically 4-5 days) and then splitted (1:5). This splitting yielded subconfluent and growing cells at day 7, the day of cell injection, at which the cells were trypsinized, washed with PBS and counted to yield a cell suspension of 10^6 cells/ml in PBS. Male C57BL mice (~20 gram each; at least 10 mice/group) were injected s.c. on the flank with a suspension of the FOR cells (100 μl/mouse). Four days later, a test compound dissolved in DMSO was injected (100 μl) i.p to the mice, twice a day (morning and evening). Each compound was injected at either 1 or 2 different concentrations (0.1 and/or 0.5 mg/mouse/day). Control mice were injected i.p. with DMSO only (100 μl). Mice were observed daily, and usually three weeks after cell injection, mice were sacrificed, the tumors were harvested and weighted.

(d) **Transmigration assay for heparanase activity**

An in vitro chamber-like transmigration system was established by using transwell filters coated with a reconstituted basement membrane-like matrix (Matrigel). Matrigel is was composed of laminin, collagen type IV, entactin and nidogen, as well as of HSPG, thus constituting a relevant heparanase substrate. The cells used in the experiment were mock-transfected Eb murine lymphoma cells not expressing heparanase and stable *hepa*-transfected Eb murine lymphoma cells overexpressing heparanase (both

cells described by Vlodavsky et al., 1999), and the migration rate of the cells trough Matrigel was evaluated first in the absence and in the presence of the chemoattractant SDF-1. Once the transmigration of the cells to the lower chamber was shown to be well correlated with the heparanase expression levels and activity, the transmigration of the Eb cells overexpressing heparanase was tested after treatment with the heparanase inhibitors of the invention. Addition of the heparanase inhibitor reduces the transmigration rate of the cells.

Example II (1). In vitro inhibition of heparanase activity by compounds of the invention.

The inhibition of heparanase activity by the compounds of the present invention was first detected in two colorimetric in vitro assays, i.e., the DMB assay and the tetrazolium blue assay as described in Methods (a) and (b) above. The human recombinant heparanase (designated h-hepa) expressed in CHO cells S1-11 subclone was used herein either in its partially purified form (50% purity) or in crude cell extracts, and the mouse recombinant heparanase (designated m-hepa) expressed in the CHO cells mhG9 clone was used herein in crude cell extracts only.

The results of the IC_{50} values of the different compounds are shown in Table 1. All the tested compounds were found to inhibit heparanase activity at micromolar concentrations. However, **Compound 2** was shown to be more potent in the DMB (h-hepa) assay with IC_{50} value of 5 μM compared to IC_{50} values in the range of 15.1 to 44.6 μM for the other compounds.

Table 1. IC_{50} values of the tested compounds for inhibition of heparanase as detected by the in vitro DMB and tetrazolium assays.

Compound	DMB (h-hepa) IC_{50}[μM]	Tetrazolium (h-hepa) IC_{50}[μM]	DMB of cell extract (h-hepa) IC_{50}[μM]	DMB of cell extract (m-hepa) IC_{50}[μM]
1	15.1	45	13	40
2	5	5.2	48	26
3	44.6			
4	19		11	15

Example II (2). Inhibition of mouse melanoma primary tumor growth by Compound 2

The effect of **Compound 2** on melanoma primary tumor growth was assayed as described in Method (c) above. The results are summarized in Table 2 below.

Table 2. Effect of Compound 2 on mouse melanoma primary tumor growth

Dose [mg/mouse/day] Tumor weight (gr)	Control	1.0
	0.75	0.1
	0.8	0.92
	1.1	0.09
	1.07	0.13
	0.86	0.19
	1.02	0.19
	0.34	0.13
	0.11	
	1.79	
	0.31	
	0.16	
Median	0.8	0.13
Range	0.16 - 1.79	0.09 - 0.92

As shown in Table 2, untreated control mice developed primary tumors with an average weight of 0.8 g. Treatment with **Compound 2** (1.0 mg/mouse/day) dramatically reduced the tumor size to 0.13 g, namely by a factor of 6.

Example II(3). Reduction of transmigration of Eb-heparanase cells by Compound 1

The effect of **Compound 1** on the transmigration of Eb murine lymphoma cells overexpressing heparanase (herein 'Eb-heparanase' cells) was assayed as described in Method (d) above. The results are summarized in Figs. 1A-B.

In the first experiment, transwell units (Costar, Cambridge, MA, USA) were coated with Matrigel (15 µl/well) and left for 8 hours at 37 °C to allow the gel to polymerize. Then, Eb murine T-lymphoma cells, mock-transfected (lacking heparanase) or heparanase-transfected (overexpressing heparanase), were plated in the transwell units (200,000 cells/well). The chemoattractant SDF-1 (PeproTech, Rocky Hill, NJ, USA) was added (250 ng/ml) to the lower chamber of the transwell units and the cells were allowed to migrate for 16 hours. Transmigration was evaluated with the CellTiter kit according to the manufacturer's instructions (Promega, Madison, WI, USA). Results are presented as % of cells migrated to the lower chamber out of the total number of cells added to the transwell unit.

As shown in Fig. 1A, plating of the mock-transfected Eb murine lymphoma cells in the absence of SDF-1 resulted in transmigration of 1.5% of cells to the lower chamber, while plating of the stable heparanase-transfected Eb cells resulted in a 5-fold increase in the transmigration rate (7.4 %). Thus, transmigration magnitude was shown to nicely correlate with the heparanase expression levels and activity. Fig. 1A also shows that transmigration of the cells was further enhanced by the chemoattractant SDF-1: 5.3 % for the mock-transfected cells and 15.7 % for the heparanase-transfected Eb cells. A three fold increase in transmigration of heparanase-transfected Eb cells was noted as compared to the control, suggesting that heparanase also contributed to the transmigration potential of cells.

Transmigration of Eb-heparanase cells treated with **Compound 1** (200 µl of a 3 mg/ml solution were added to the cells in the upper chamber) was then tested. As shown

in Fig. 1B, **Compound 1** reduced transmigration of the Eb-heparanase cells by about 70%.

REFERENCES

Eastmond, G. C., Paprotny, J., (1998) Methyl- and fluoro-substituted bis(4-aminophenoxy) benzenes. A convenient method of synthesis. Synthesis, 6: 894-898.

Kawase, Y., Takahashi, M., Takatsu, T., Arai, M., Nakajima, M., and Tanzawa, K. (1995) A-72363 A-1,A-2, and C, novel heparanase inhibitors from *Streptomyces nobilis* SANK 60192. II. Biological activities. J. Antibiotics 49: 61-64.

Lapierre, F., Holme, K., Lam, L., Tressler, R.J., Storm, N., Wee, J., Stack, R.J., Casrellot, J., Tyrrell, D.J. (1996) Chemical modifications of heparin that diminish its anticoagulant but preserve its heparanase-inhibitory, angiostatic, anti-tumor and anti-metastatic properties. Glycobiol. 6: 355-366.

Lider, O., Baharav, E., Mekori, Y.A., Miller, T., Naparstek, Y., Vlodavsky, I., and Cohen, I.R. (1989) Suppression of experimental autoimmune diseases and prolongation of allograft survival by treatment of animals with heparinoid inhibitors of T lymphocyte heparanase. J. Clin. Invest. 83: 752-756.

Nakajima, M., DeChavigny A., Johnson, C.E., Hamada, J-I, Stein, C.A., and Nicolson, G.L. (1991) Suramin a potent inhibitor of melanoma heparanase and invasion. J. Biol. Chem. 266: 9661-9666.

Nakajima, M., Irimura, T., and Nicolson, G.L. (1988) Heparanase and tumor metastasis. J. Cell. Biochem. 36: 157-167.

Nakajima, M., Irimura, T., Di Ferrante, N., and Nicolson, G.L (1984) Metastatic melanoma cell heparanase. Characterization of heparan sulfate degradation fragments produced by B16 melanoma endoglucuronidase J. Biol. Chem. 259: 2283-2290. Nicosia, R.F., Lin, Y.J., Hazelton, D., and Qian, X. (1997) Endogenous regulation of angiogenesis in the rat aorta model. Amer. J. Pathol. 151: 1379-1386.

Nicosia, R.F., and Ottinetti, A. (1990) Growth of microvessels in serum-free matrix culture of rat aorta: a quantitative assay of angiogenesis in vitro. Lab. Invest. 63: 115-122.

Nishimura, Y., Kudo, T., Kondo, S., Takeuchi, T., Tsuruoka, T., Fukuyasu, H., and Shibahara, S. (1994) Totally synthetic analogs of siastatin B. III. Trifluoroacetamide analogs having inhibitory activity for tumor metastasis. J. Antibiot. 47: 101-107.

Parish, C.R., Coombe, D.R., Jackson, K.B., and Underwood P.A. (1987) Evidence that sulfated polysaccharides inhibit tumor metastasis by blocking tumor cell-derived heparanase. Int. J. Cancer 40: 511-517.

Parish, C.R., Freeman, C., Brown, K.J., Francis, D.J., and Cowden, W.B. (1999) Identification of sulfated oligosaccharide-based inhibitors of tumor growth and metastasis using novel in vitro assays for angiogenesis and heparanase activity. Cencer Res. 59: 3433-3441.

Shevelev, S. A., Dutov, M. D., Vatsadze, I. A., Serushkina, O. V., Korelev, M. A., Rusanov, A. L. (1995) Phenol substitution of nitro groups in 1,3,5-trinitrobenzene - method of preparation of 5-nitroresorcinol diaryl ethers and 3,5-dinitrophenyl aryl ethers. Izv. Akad. Nauk, Ser. Khim. 2: 393-394.

Vlodavsky, I., Friedmann, Y., Elkin, M., Aingorn, H., Atzmon, R., Ishai-Michaeli, R., Bitan, M., Pappo, O., Peretz, T., Michal, I., Spector, L., and Pecker, I. (1999). Mammalian heparanase: Gene cloning, expression and function in tumor progression and metastasis. Nat. Med. 5: 793-802.

Vlodavsky, I., Hua-Quan Miao., Benezra, M., Lider, O., Bar-Shavit, R., Schmidt, A., and Peretz, T. (1997). Involvement of the extracellular matrix, heparan sulfate proteoglycans and heparan sulfate degrading enzymes in angiogenesis and metastasis. In: Tumor Angiogenesis. Eds. C. E. Lewis, R. Bicknell & N. Ferrara. Oxford University Press, Oxford UK, pp. 125-140.

Vlodavsky, I., Mohsen, M., Lider, O., Svahn, C.M., Ekre, H.P., Vigoda, M., Ishai-Michaeli, R., and Peretz, T. (1994) Inhibition of tumor metastasis by heparanase inhibiting species of heparin. Invasion Metastasis14:290-302.

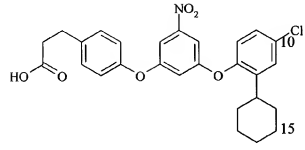
Vlodavsky, I., Eldor, A., Haimovitz-Freidman, A., Matzner, Y., Ishai-Michaeli, R., Levi, E., Bashkin, P., Lider, O. Naparstek, Y., Cohen, I.R., and Fuks, Z. (1992) Expression of heparanase by platelets and circulating cells of the immune system: Possible involvement in diapedesis and extravasation. Invasion Metastasis 12: 112-127.

Vlodavsky, I., Ishai-Michaeli, R., Bar-Ner, M., Freidman, R., Horowitz, A.T., Fuks, Z., and Biran, S. (1988) Involvement of heparanase in tumor metastasis and angiogenesis. Isr. J. Med. 24: 464-470.

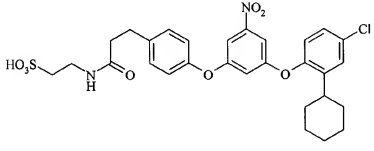
Vlodavsky, I., Fuks, Z., Bar-Ner, M., Ariav, Y., and Schirrmacher, V. (1983) Lymphoma cell mediated degradation of sulfated proteoglycans in the subendothelial extracellular matrix: Relationship to tumor cell metastasis. Cancer Res. 43: 2704-2711.

Appendix A- Compounds 1-4

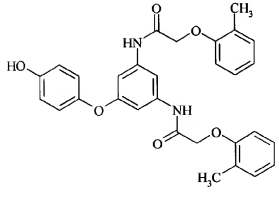
Compound 1



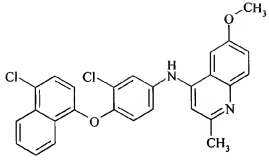
Compound 2



Compound 3



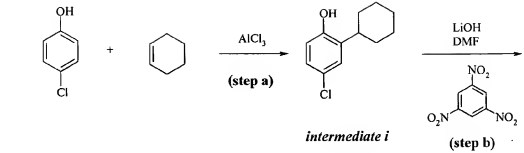
Compound 4



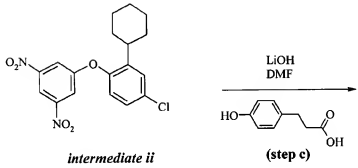
Appendix B- SCHEMES

Scheme 1

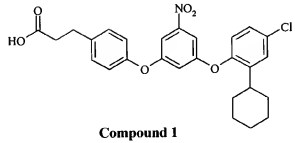
Scheme 2



intermediate i

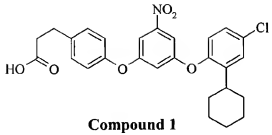


intermediate ii



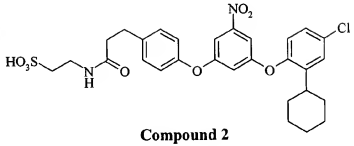
Compound 1

Scheme 3



Compound 1

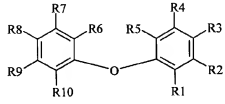
taurine, EEDQ



Compound 2

CLAIMS

1. A pharmaceutical composition for treatment of diseases and disorders caused by or associated with heparanase catalytic activity, said composition comprising a pharmaceutical acceptable carrier and a heparanase inhibitor which is a diphenyl ether of the Formula I:



(I)

wherein

R1, R5, R6 and R7 each independently represents hydrogen or halogen;

R2, R3, R4 and R8 each independently represents hydrogen, halogen, nitro, -OR', -SR', -NR11R12, -COOR', -CONR11R12, -SO_3H, -SO_2NR11R12, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, C6-C14 aryl or heteroaryl;

R9 and R10 each independently is hydrogen or halogen, or R9 and R10 together with the carbon atoms to which they are attached form a condensed benzene ring;

R11 and R12, each independently represents hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or heteroaryl;

or R11 is H and R12 is C2-C7 alkanoyl or C7-C15 aroyl, or R11 and R12 together with the N atom to which they are attached form a saturated 5-7 membered heterocyclic ring containing one to three heteroatoms selected from N, O and/or S;

R' is hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or heteroaryl;

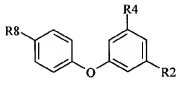
"heteroaryl" in radicals R2, R3, R4, R8, R11, R12 and R' is a radical derived from a mono- or poly-cyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

any "C1-C6 alkyl", "C2-C7 alkanoyl" and C2-C6 alkenyl in radicals R2, R3, R4, R8 R11, R12 and R' may be substituted by at least one group selected from halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, -SO_3H, -SO_2NR11R12, C6-C14 aryl, and heteroaryl;

any "C6-C14 aryl", "C7-C15 aroyl" and "heteroaryl" in radicals R2, R3, R4, R8 R11, R12 and R' may be substituted by at least one group selected from halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, and C5-C6 cycloalkyl;

and pharmaceutically acceptable salts thereof.

2. A pharmaceutical composition according to Claim 1 comprising a compound of the formula Ia:

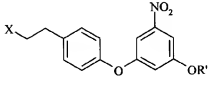


(Ia)

wherein R2, R4 and R8 are as defined in Claim 1.

3. A pharmaceutical composition according to Claim 2, wherein R2 is –OR', R4 is nitro, and R8 is C1-C6 alkyl optionally substituted by at least one group selected from halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C6-C14 aryl, and heteroaryl, wherein R', R11 and R12 are as defined in Claim 1.

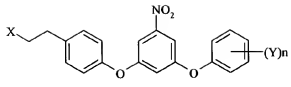
4. A pharmaceutical composition according to Claim 3 comprising a compound of the formula Ib:



(Ib)

wherein X is -COOR' or -CONR11R12, wherein R', R11 and R12 are as defined in Claim 1.

5. A pharmaceutical composition according to Claim 4 comprising a compound of the formula Ic:

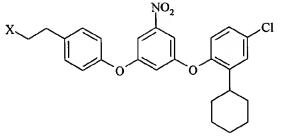


(Ic)

wherein Y is halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, -SO_3H, -$SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl, n is an integer from 0 to 5, X is as defined in Claim 4, and R', R11 and R12 are as defined in Claim 1.

6. A pharmaceutical composition according to Claim 5, wherein n is 2, one Y is halogen and another Y is C5-C6 cycloalkyl, and X is as defined in Claim 4.

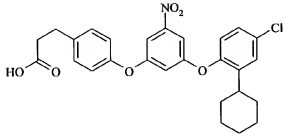
7. A pharmaceutical composition according to Claim 6 comprising a compound of the formula Id:



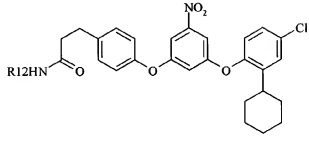
(Id)

wherein X is -COOR' or –CONR11R12, wherein R' and R11 are hydrogen and R12 is C1-C6 alkyl optionally substituted by halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, -SO_3H, -$SO_2NR11R12$, C6-C14 aryl, or heteroaryl.

8. A pharmaceutical composition according to Claim 7 comprising the compound herein designated **Compound 1** of the formula:



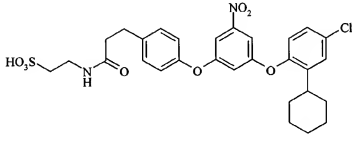
9. A pharmaceutical composition according to Claim 7 comprising a compound of the formula Ie:



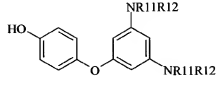
(Ie)

wherein R12 is C1-C6 alkyl optionally substituted by halogen, -OR', -SR', -COOR', nitro, $-SO_3H$, C6-C14 aryl, or heteroaryl, wherein R' is as defined in Claim 1.

10. A pharmaceutical composition according to Claim 9 comprising the compound herein designated **Compound 2** of the formula:



11. A pharmaceutical composition according to Claim 2 comprising a compound of the formula If:



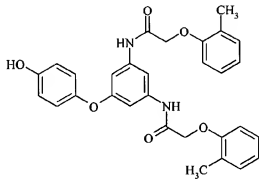
(If)

wherein R11 and R12 are as defined in Claim 1.

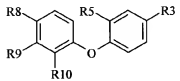
12. A pharmaceutical composition according to Claim 11, wherein R11 is hydrogen and R12 is C2-C7 alkanoyl optionally substituted by halogen, -OR', -SR', -COOR', nitro, $-SO_3H$, C6-C14 aryl, and heteroaryl; or C7-C15 aroyl optionally substituted by at least one group selected from halogen, -OR', -SR', -COOR', nitro, $-SO_3H$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, and C5-C6 cycloalkyl, wherein R' is as defined in Claim 1.

13. A pharmaceutical composition according to Claim 12, wherein R12 is C2-C7 alkanoyl optionally substituted by –OR', wherein R' is a C6-C14 aryl substituted by C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl.

14. A pharmaceutical composition according to Claim 13 comprising the compound herein designated **Compound 3** of the formula:



15. A pharmaceutical composition according to Claim 1 comprising a compound of the formula Ig:

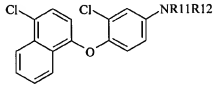


(Ig)

wherein R3, R5, R8, R9 and R10 are as defined in Claim 1.

16. A pharmaceutical composition according to Claim 15, wherein R3 is -NR11R12, R5 and R8 are halogen, R9 and R10 together with the carbon atoms to which they are attached form a condensed benzene ring, and wherein R11 and R12 are as defined in Claim 1.

17. A pharmaceutical composition according to Claim 16 comprising a compound of the formula Ih:

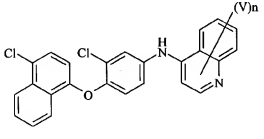


(Ih)

wherein R11 and R12 are as defined in Claim 1.

18. A pharmaceutical composition according to Claim 17, wherein R11 is hydrogen and R12 is C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or heteroaryl optionally substituted by halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl, wherein R' is as defined in Claim 1.

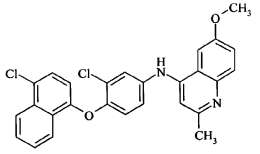
19. A pharmaceutical composition according to Claim 18 comprising a compound of the formula Ii:



(Ii)

wherein V is halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl, n is an integer from 0 to 6, and R', R11 and R12 are as defined in Claim 1.

20. A pharmaceutical composition according to Claim 19 comprising the compound herein designated **Compound 4** of the formula:



21. A pharmaceutical composition according to any one of claims 1 to 20 for inhibition of angiogenesis.

22. A pharmaceutical composition according to any one of claims 1 to 20 for treatment or inhibition of a malignant cell proliferative disease or disorder.

23. The pharmaceutical composition according to claim 21 or 22 for the treatment or inhibition of non-solid cancers, e.g hematopoietic malignancies such as all types of leukemia, e.g. acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), myelodysplastic syndrome (MDS), mast cell leukemia, hairy cell leukemia, Hodgkin's disease, non-Hodgkin's lymphomas, Burkitt's lymphoma and multiple myeloma.

24. The pharmaceutical composition according to claim 21 or 22 for the treatment or inhibition of solid tumors such as tumors in lip and oral cavity, pharynx, larynx, paranasal sinuses, major salivary glands, thyroid gland, esophagus, stomach, small intestine, colon, colorectum, anal canal, liver, gallbladder, extrahepatic bile ducts, ampulla of vater, exocrine pancreas, lung, pleural mesothelioma, bone, soft tissue sarcoma, carcinoma and malignant melanoma of the skin, breast, vulva, vagina, cervix uteri, corpus uteri, ovary, fallopian tube, gestational trophoblastic tumors, penis, prostate, testis, kidney, renal pelvis, ureter, urinary bladder, urethra, carcinoma of the eyelid, carcinoma of the

conjunctiva, malignant melanoma of the conjunctiva, malignant melanoma of the uvea, retinoblastoma, carcinoma of the lacrimal gland, sarcoma of the orbit, brain, spinal cord, vascular system, hemangiosarcoma and Kaposi's sarcoma.

25. The pharmaceutical composition according to claim 23 or 24 for treating or inhibiting tumor formation, primary tumors, tumor progression or tumor metastasis.

26. A pharmaceutical composition according to any one of claims 1 to 21 for treatment of ophthalmologic disorders such as diabetic retinopathy and macular degeneration, particularly age-related macular degeneration.

27. The pharmaceutical composition according to any one of claims 1 to 20 for inhibiting or treating cell proliferative diseases or disorders such as psoriasis, hypertrophic scars, acne and sclerosis/scleroderma.

28. The pharmaceutical composition according to any one of claims 1 to 20 for inhibiting or treatment of a disease or disorder selected from polyps, multiple exostosis, hereditary exostosis, retrolental fibroplasia, hemangioma, reperfusion of gastric ulcer and arteriovenous malformation.

29. The pharmaceutical composition according to any one of claims 1 to 20, for contraception or for inducing abortion at early stages of pregnancy.

30. The pharmaceutical composition according to any one of claims 1 to 20, for treatment of or amelioration of inflammatory symptoms in any disease, condition or disorder where immune and/or inflammation suppression is beneficial.

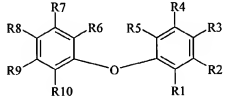
31. The pharmaceutical composition according to claim 30, for treatment of or amelioration of inflammatory symptoms in the joints, musculoskeletal and connective tissue disorders.

32. The pharmaceutical composition according to claim 30, for treatment of or amelioration of inflammatory symptoms associated with hypersensitivity, allergic reactions, asthma, atherosclerosis, otitis and other otorhinolaryngological diseases, dermatitis and other skin diseases, posterior and anterior uveitis, conjunctivitis, optic neuritis, scleritis and other immune and/or inflammatory ophthalmic diseases.

33. The pharmaceutical composition according to any one of claims 1 to 20, for treatment of or amelioration of an autoimmune disease.

34. The pharmaceutical composition according to claim 33, wherein said autoimmune disease is Eaton-Lambert syndrome, Goodpasture's syndrome, Grave's disease, Guillain-Barré syndrome, autoimmune hemolytic anemia (AIHA), hepatitis, insulin-dependent diabetes mellitus (IDDM), systemic lupus erythematosus (SLE), multiple sclerosis (MS), myasthenia gravis, plexus disorders e.g. acute brachial neuritis,, polyglandular deficiency syndrome, primary biliary cirrhosis, rheumatoid arthritis, scleroderma, thrombocytopenia, thyroiditis e.g. Hashimoto's disease, Sjögren's syndrome, allergic purpura, psoriasis, mixed connective tissue disease, polymyositis, dermatomyositis, vasculitis, polyarteritis nodosa, polymyalgia rheumatica, Wegener's granulomatosis, Reiter's syndrome, Behçet's syndrome, ankylosing spondylitis, pemphigus, bullous pemphigoid, dermatitis herpetiformis, Crohn's disease or autism.

35. Use of a heparanase inhibitor or of a pharmaceutically acceptable salt thereof, wherein said heparanase inhibitor is a diphenyl ether compound of the Formula I:



(I)

wherein

R1, R5, R6 and R7 each independently represents hydrogen or halogen;

R2, R3, R4 and R8 each independently represents hydrogen, halogen, nitro, -OR', -SR', -NR11R12, -COOR', -CONR11R12, -SO_3H, -SO_2NR11R12, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, C6-C14 aryl or heteroaryl;

R9 and R10 each independently is hydrogen or halogen, or R9 and R10 together with the carbon atoms to which they are attached, form a condensed benzene ring;

R11 and R12 each independently represents hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or heteroaryl;

or R11 is H and R12 is C2-C7 alkanoyl or C7-C15 aroyl, or R11 and R12 together with the N atom to which they are attached form a saturated 5-7 membered heterocyclic ring containing one to three heteroatoms selected from N, O and/or S;

R' is hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or heteroaryl;

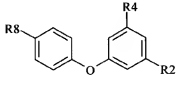
"heteroaryl" in radicals R2, R3, R4, R8, R11, R12 and R' is a radical derived from a mono- or poly-heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

any "C1-C6 alkyl", "C2-C7 alkanoyl" and "C2-C6 alkenyl" in radicals R2, R3, R4, R8 R11, R12 and R' may be substituted by at least one group selected from halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, -SO_3H, -SO_2NR11R12, C6-C14 aryl, and heteroaryl;

any "C6-C14" aryl, "C7-C15" aroyl and "heteroaryl" in radicals R2, R3, R4, R8 R11, R12 and R' may be substituted by at least one group selected from halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, -SO_3H, -SO_2NR11R12, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, and C5-C6 cycloalkyl;

for the preparation of a pharmaceutical composition for treatment of a disease or a disorder caused by or associated with heparanase catalytic activity.

36. Use according to Claim 35 of a compound of the formula Ia:

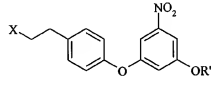


(Ia)

wherein R2, R4 and R8 are as defined in Claim 35.

37. Use according to Claim 36, wherein R2 is –OR', R4 is nitro, and R8 is C1-C6 alkyl optionally substituted by at least one group selected from halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C6-C14 aryl, and heteroaryl, wherein R', R11 and R12 are as defined in Claim 35.

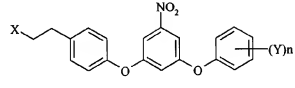
38. Use according to Claim 37 of a compound of the formula Ib:



(Ib)

wherein X is -COOR' or -CONR11R12, wherein R', R11 and R12 are as defined in Claim 35.

39. Use according to Claim 38 of a compound of the formula Ic:

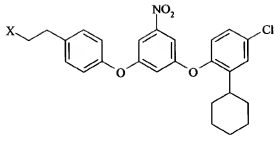


(Ic)

wherein Y is halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl, n is an integer from 0 to 5, X is as defined in Claim 4, and R', R11 and R12 are as defined in Claim 35.

40. Use according to Claim 39, wherein n is 2, one Y is halogen and another Y is C5-C6 cycloalkyl, and X is as defined in Claim 38.

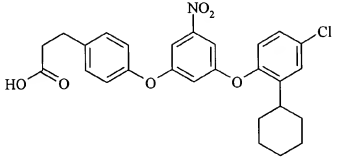
41. Use according to Claim 40 of a compound of the formula Id:



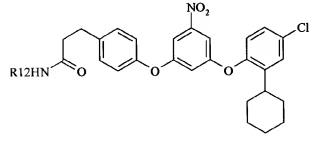
(Id)

wherein X is -COOR' or –CONR11R12, wherein R' and R11 are hydrogen and R12 is C1-C6 alkyl optionally substituted by halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C6-C14 aryl, or heteroaryl.

42. Use according to Claim 41 of the compound herein designated **Compound 1** of the formula:



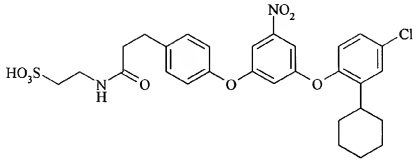
43. Use according to Claim 41 of a compound of the formula Ie:



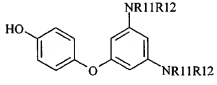
(Ie)

wherein R12 is C1-C6 alkyl optionally substituted by halogen, -OR', -SR', -COOR', nitro, $-SO_3H$, C6-C14 aryl, or heteroaryl, wherein R' is as defined in Claim 35.

44. Use according to Claim 43 of the compound herein designated **Compound 2** of the formula:



45. Use according to Claim 36 of a compound of the formula If:



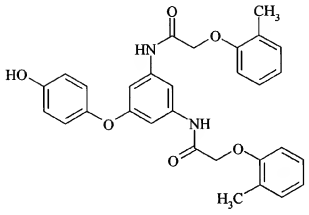
(If)

wherein R11 and R12 are as defined in Claim 35.

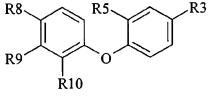
46. Use according to Claim 45, wherein R11 is hydrogen and R12 is C2-C7 alkanoyl optionally substituted by halogen, -OR', -SR', -COOR', nitro, $-SO_3H$, C6-C14 aryl, and heteroaryl; or C7-C15 aroyl optionally substituted by at least one group selected from halogen, -OR', -SR', -COOR', nitro, $-SO_3H$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, and C5-C6 cycloalkyl, and R' is as defined in Claim 35.

47. Use according to Claim 46, wherein R12 is C2-C7 alkanoyl optionally substituted by –OR', wherein R' is a C6-C14 aryl substituted by C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl.

48. Use according to Claim 47 of the compound herein designated **Compound 3** of the formula:



49. Use according to Claim 35 of a compound of the formula Ig:

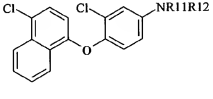


(Ig)

wherein R3, R5, R8, R9 and R10 are as defined in Claim 35.

50. Use according to Claim 49, wherein R3 is -NR11R12, R5 and R8 are halogen, R9 and R10 together with the carbon atoms to which they are attached form a condensed benzene ring, and wherein R11 and R12 are as defined in Claim 35.

51. Use according to Claim 50 of a compound of the formula Ih:

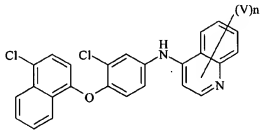


(Ih)

wherein R11 and R12 are as defined in Claim 35.

52. Use according to Claim 51, wherein R11 is hydrogen and R12 is C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or heteroaryl optionally substituted by halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl, wherein R' is as defined in Claim 35.

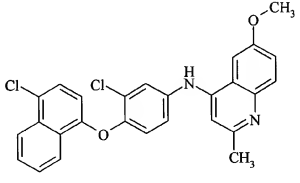
53. Use according to Claim 52 of a compound of the formula Ii:



(Ii)

wherein V is halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl, n is an integer from 0 to 6, and R', R11 and R12 are as defined in Claim 35.

54. Use according to Claim 53 of the compound herein designated **Compound 4** of the formula:



55. Use according to any one of claims 35 to 54 for the preparation of a pharmaceutical composition for inhibition of angiogenesis.

56. Use according to any one of claims 35 to 54 for the preparation of a pharmaceutical composition for treatment or inhibition of a malignant cell proliferative disease or disorder.

57. Use according to claim 55 or 56 for the preparation of a pharmaceutical composition for the treatment or inhibition of non-solid cancers, e.g hematopoietic malignancies such as all types of leukemia, e.g. acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), myelodysplastic syndrome (MDS), mast cell leukemia, hairy cell leukemia, Hodgkin's disease, non-Hodgkin's lymphomas, Burkitt's lymphoma and multiple myeloma.

58. Use according to claim 55 or 56 for the preparation of a pharmaceutical composition for the treatment or inhibition of solid tumors such as tumors in lip and oral cavity, pharynx, larynx, paranasal sinuses, major salivary glands, thyroid gland, esophagus, stomach, small intestine, colon, colorectum, anal canal, liver, gallbladder, extrahepatic bile ducts, ampulla of vater, exocrine pancreas, lung, pleural mesothelioma, bone, soft tissue sarcoma, carcinoma and malignant melanoma of the skin, breast, vulva, vagina, cervix uteri, corpus uteri, ovary, fallopian tube, gestational trophoblastic tumors, penis, prostate, testis, kidney, renal pelvis, ureter, urinary bladder, urethra, carcinoma of the eyelid, carcinoma of the conjunctiva, malignant melanoma of the conjunctiva, malignant melanoma of the uvea, retinoblastoma, carcinoma of the lacrimal gland, sarcoma of the orbit, brain, spinal cord, vascular system, hemangiosarcoma and Kaposi's sarcoma.

59. Use according to claim 57 or 58 for the preparation of a pharmaceutical composition for treating or inhibiting tumor formation, primary tumors, tumor progression or tumor metastasis.

60. Use according to any one of claims 35 to 54 for the preparation of a pharmaceutical composition for treatment of ophthalmologic disorders such as diabetic retinopathy and macular degeneration, particularly age-related macular degeneration.

61. Use according to any one of claims 35 to 54 for the preparation of a pharmaceutical composition for inhibiting or treating cell proliferative diseases or disorders such as psoriasis, hypertrophic scars, acne and sclerosis/scleroderma.

62. Use according to any one of claims 35 to 54 for the preparation of a pharmaceutical composition for inhibiting or treatment of a disease or disorder selected from polyps, multiple exostosis, hereditary exostosis, retrolental fibroplasia, hemangioma, reperfusion of gastric ulcer and arteriovenous malformation.

63. Use according to any one of claims 35 to 54 for the preparation of a pharmaceutical composition for contraception or for inducing abortion at early stages of pregnancy.

64. Use according to any one of claims 35 to 54 for the preparation of a pharmaceutical composition for treatment of or amelioration of inflammatory symptoms in any disease, condition or disorder where immune and/or inflammation suppression is beneficial.

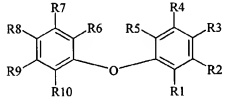
65. Use according to claim 64, wherein said pharmaceutical composition is for treatment of or amelioration of inflammatory symptoms in the joints, musculoskeletal and connective tissue disorders.

66. Use according to claim 64, wherein said pharmaceutical composition is for treatment of or amelioration of inflammatory symptoms associated with hypersensitivity, allergic reactions, asthma, atherosclerosis, otitis and other otorhinolaryngological diseases, dermatitis and other skin diseases, posterior and anterior uveitis, conjunctivitis, optic neuritis, scleritis and other immune and/or inflammatory ophthalmic diseases.

67. Use according to any one of claims 35 to 54 for the preparation of a pharmaceutical composition for treatment of or amelioration of an autoimmune disease.

68. Use according to claim 67 wherein said autoimmune disease is Eaton-Lambert syndrome, Goodpasture's syndrome, Grave's disease, Guillain-Barré syndrome, autoimmune hemolytic anemia (AIHA), hepatitis, insulin-dependent diabetes mellitus (IDDM), systemic lupus erythematosus (SLE), multiple sclerosis (MS), myasthenia gravis, plexus disorders e.g. acute brachial neuritis, polyglandular deficiency syndrome, primary biliary cirrhosis, rheumatoid arthritis, scleroderma, thrombocytopenia, thyroiditis e.g. Hashimoto's disease, Sjögren's syndrome, allergic purpura, psoriasis, mixed connective tissue disease, polymyositis, dermatomyositis, vasculitis, polyarteritis nodosa, polymyalgia rheumatica, Wegener's granulomatosis, Reiter's syndrome, Behçet's syndrome, ankylosing spondylitis, pemphigus, bullous pemphigoid, dermatitis herpetiformis, Crohn's disease or autism.

69. A method for treatment of a patient suffering from a disease or disorder caused by or associated with heparanase catalytic activity, which comprises administering to said patient an effective amount of a heparanase inhibitor or a pharmaceutically acceptable salt thereof, wherein said heparanase inhibitor is a diphenyl ether of the Formula I:



(I)

wherein

R1, R5, R6 and R7 each independently represents hydrogen or halogen;

R2, R3, R4 and R8 each independently represents hydrogen, halogen, nitro, -OR', -SR', -NR11R12, -COOR', -CONR11R12, -SO_3H, -SO_2NR11R12, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, C6-C14 aryl or heteroaryl;

R9 and R10 each independently is hydrogen or halogen, or R9 and R10 together with the carbon atoms to which they are attached, form a condensed benzene ring;

R11 and R12 each independently represents hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or heteroaryl;

or R11 is H and R12 is C2-C7 alkanoyl or C7-C15 aroyl, or R11 and R12 together with the N atom to which they are attached form a saturated 5-7 membered heterocyclic ring containing one to three heteroatoms selected from N, O and/or S;

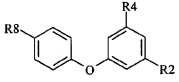
R' is hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or heteroaryl;

"heteroaryl" in radicals R2, R3, R4, R8, R11, R12 and R' is a radical derived from a mono- or poly-heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

any "C1-C6 alkyl", "C2-C7 alkanoyl" and "C2-C6 alkenyl" in radicals R2, R3, R4, R8 R11, R12 and R' may be substituted by at least one group selected from halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C6-C14 aryl, and heteroaryl; and

any "C6-C14 aryl", "C7-C15 aroyl" and "heteroaryl" in radicals R2, R3, R4, R8 R11, R12 and R' may be substituted by at least one group selected from halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, and C5-C6 cycloalkyl.

70. A method according to Claim 69, wherein the heparanase inhibitor is a compound of the formula Ia:

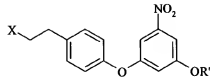


(Ia)

wherein R2, R4 and R8 are as defined in Claim 69.

71. A method according to Claim 70, wherein R2 is –OR', R4 is nitro, and R8 is C1-C6 alkyl optionally substituted by at least one group selected from halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C6-C14 aryl, and heteroaryl, wherein R', R11 and R12 are as defined in Claim 69.

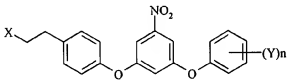
72. A method according to Claim 71, wherein the heparanase inhibitor is a compound of the formula Ib:



(Ib)

wherein X is -COOR' or -CONR11R12, wherein R', R11 and R12 are as defined in Claim 69.

73. A method according to Claim 40, wherein the heparanase inhibitor is a compound of the formula Ic:

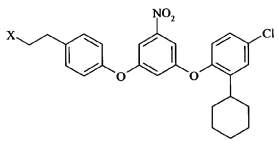


(Ic)

wherein Y is halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, -SO_3H, -$SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl, n is an integer from 0 to 5, X is as defined in Claim 72, and R', R11 and R12 are as defined in Claim 69.

74. A method according to Claim 73, wherein n is 2, one Y is halogen and another Y is C5-C6 cycloalkyl, and X is as defined in Claim 72.

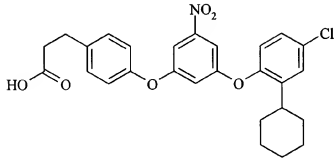
75. A method according to Claim 74, wherein the heparanase inhibitor is a compound of the formula Id:



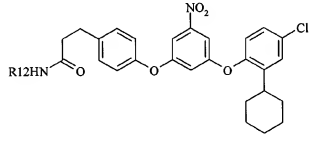
(Id)

wherein X is -COOR' or –CONR11R12, wherein R' and R11 are hydrogen and R12 is C1-C6 alkyl optionally substituted by halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C6-C14 aryl, or heteroaryl.

76. A method according to Claim 75, wherein the heparanase inhibitor is the compound herein designated **Compound 1** of the formula:



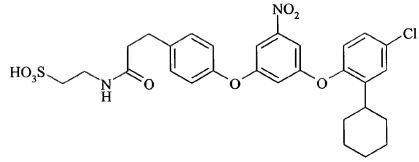
77. A method according to Claim 75, wherein the heparanase inhibitor is a compound of the formula Ie:



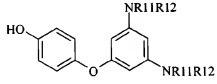
(Ie)

wherein R12 is C1-C6 alkyl optionally substituted by halogen, -OR', -SR', -COOR', nitro, $-SO_3H$, C6-C14 aryl, or heteroaryl, wherein R' is as defined in Claim 69.

78. A method according to Claim 77, wherein the heparanase inhibitor is the compound herein designated **Compound 2** of the formula:



79. A method according to Claim 70, wherein the heparanase inhibitor is a compound of the formula If:



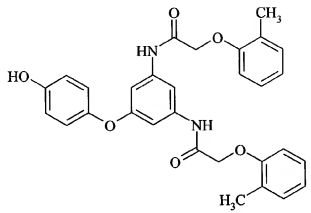
(If)

wherein R11 and R12 are as defined in Claim 69.

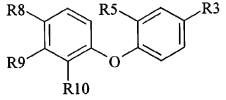
80. A method according to Claim 79, wherein R11 is hydrogen and R12 is C2-C7 alkanoyl optionally substituted by halogen, -OR', -SR', -COOR', nitro, $-SO_3H$, C6-C14 aryl, and heteroaryl; or C7-C15 aroyl optionally substituted by at least one group selected from halogen, -OR', -SR', -COOR', nitro, $-SO_3H$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, and C5-C6 cycloalkyl, wherein R' is as defined in Claim 69.

81. A method according to Claim 80, wherein R12 is C2-C7 alkanoyl optionally substituted by –OR', wherein R' is a C6-C14 aryl substituted by C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl.

82. A method according to Claim 81, wherein the heparanase inhibitor is the compound herein designated **Compound 3** of the formula:



83. A method according to Claim 69, wherein the heparanase inhibitor is a compound of the formula Ig:

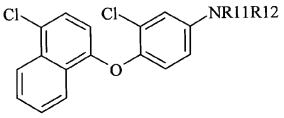


(Ig)

wherein R3, R5, R8, R9 and R10 are as defined in Claim 69.

84. A method according to Claim 83, wherein R3 is -NR11R12, R5 and R8 are halogen, R9 and R10 together with the carbon atoms to which they are attached form a condensed benzene ring, and wherein R11 and R12 are as defined in Claim 69.

85. A method according to Claim 84, wherein the heparanase inhibitor is a compound of the formula Ih:

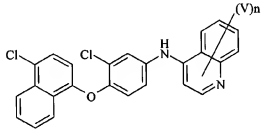


(Ih)

wherein R11 and R12 are as defined in Claim 69.

86. A method according to Claim 85, wherein R11 is hydrogen and R12 is C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or heteroaryl optionally substituted by halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl, wherein R' is as defined in Claim 69.

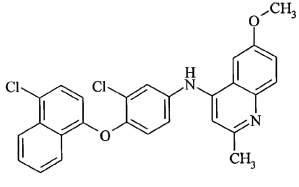
87. A method according to Claim 86, wherein the heparanase inhibitor is a compound of the formula Ii:



(Ii)

wherein V is halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl, n is an integer from 0 to 6, and R', R11 and R12 are as defined in Claim 69.

88. A method according to Claim 87, wherein the heparanase inhibitor is the compound herein designated **Compound 4** of the formula:



89. A method according to any one of claims 69 to 88 for inhibition of angiogenesis.

90. A method according to any one of claims 69 to 88 for treatment or inhibition of a malignant cell proliferative disease or disorder.

91. A method according to claim 89 or 90 for the treatment or inhibition of a non-solid cancer, e.g a hematopoietic malignancy such as any type of leukemia, e.g. acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), myelodysplastic syndrome (MDS), mast cell leukemia, hairy cell leukemia, Hodgkin's disease, non-Hodgkin's lymphomas, Burkitt's lymphoma and multiple myeloma.

92. A method according to claim 89 or 90 for the treatment or inhibition of solid tumors such as tumors in lip and oral cavity, pharynx, larynx, paranasal sinuses, major salivary glands, thyroid gland, esophagus, stomach, small intestine, colon, colorectum, anal canal, liver, gallbladder, extrahepatic bile ducts, ampulla of vater, exocrine pancreas, lung, pleural mesothelioma, bone, soft tissue sarcoma, carcinoma and malignant melanoma of the skin, breast, vulva, vagina, cervix uteri, corpus uteri, ovary, fallopian tube, gestational trophoblastic tumors, penis, prostate, testis, kidney, renal pelvis, ureter, urinary bladder, urethra, carcinoma of the eyelid, carcinoma of the conjunctiva, malignant melanoma of the conjunctiva, malignant melanoma of the uvea, retinoblastoma, carcinoma of the lacrimal gland, sarcoma of the orbit, brain, spinal cord, vascular system, hemangiosarcoma and Kaposi's sarcoma.

93. A method according to claim 91 or 92 for treating or inhibiting tumor formation, primary tumors, tumor progression or tumor metastasis.

94. A method according to any one of claims 69 to 88 for treatment of ophthalmologic disorders such as diabetic retinopathy and macular degeneration, particularly age-related macular degeneration.

95. A method according to any one of claims 69 to 88 for inhibiting or treating cell proliferative diseases or disorders such as psoriasis, hypertrophic scars, acne and sclerosis/scleroderma.

96. A method according to any one of claims 69 to 88 for inhibiting or treatment of a disease or disorder selected from polyps, multiple exostosis, hereditary exostosis, retrolental fibroplasia, hemangioma, reperfusion of gastric ulcer and arteriovenous malformation.

97. A method according to any one of claims 69 to 88 for contraception or for inducing abortion at early stages of pregnancy.

98. A method according to any one of claims 69 to 88 for treatment of or amelioration of inflammatory symptoms in any disease, condition or disorder where immune and/or inflammation suppression is beneficial.

99. A method according to claim 98, for treatment of or amelioration of inflammatory symptoms in the joints, musculoskeletal and connective tissue disorders.

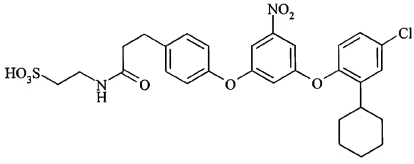
100. A method according to claim 98, for treatment of or amelioration of inflammatory symptoms associated with hypersensitivity, allergic reactions, asthma, atherosclerosis, otitis and other otorhinolaryngological diseases, dermatitis and other skin diseases, posterior and anterior uveitis, conjunctivitis, optic neuritis, scleritis and other immune and/or inflammatory ophthalmic diseases.

101. A method according to any one of claims 69 to 88 for treatment of or amelioration of an autoimmune disease.

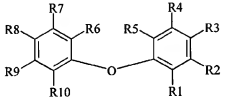
102. A method according to claim 101 wherein said autoimmune disease is Eaton-Lambert syndrome, Goodpasture's syndrome, Grave's disease, Guillain-Barré syndrome, autoimmune hemolytic anemia (AIHA), hepatitis, insulin-dependent diabetes mellitus (IDDM), systemic lupus erythematosus (SLE), multiple sclerosis (MS), myasthenia

gravis, plexus disorders e.g. acute brachial neuritis, polyglandular deficiency syndrome, primary biliary cirrhosis, rheumatoid arthritis, scleroderma, thrombocytopenia, thyroiditis e.g. Hashimoto's disease, Sjögren's syndrome, allergic purpura, psoriasis, mixed connective tissue disease, polymyositis, dermatomyositis, vasculitis, polyarteritis nodosa, polymyalgia rheumatica, Wegener's granulomatosis, Reiter's syndrome, Behçet's syndrome, ankylosing spondylitis, pemphigus, bullous pemphigoid, dermatitis herpetiformis, Crohn's disease or autism.

103. The compound herein designated **Compound 2** of the formula:



104. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and at least one diphenyl ether of the Formula I:



(I)

wherein

R1, R5, R6 and R7 each independently represents hydrogen or halogen;

R2, R3, R4 and R8 each independently represents hydrogen, halogen, nitro, -OR', -SR', -NR11R12, -COOR', -CONR11R12, -SO_3H, -SO_2NR11R12, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, C6-C14 aryl or heteroaryl;

R9 and R10 each independently is hydrogen or halogen, or R9 and R10 together with the carbon atoms to which they are attached form a condensed benzene ring;

R11 and R12, each independently represents hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or heteroaryl;

or R11 is H and R12 is C2-C7 alkanoyl or C7-C15 aroyl, or R11 and R12 together with the N atom to which they are attached form a saturated 5-7 membered heterocyclic ring containing one to three heteroatoms selected from N, O and/or S;

R' is hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or heteroaryl;

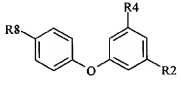
"heteroaryl" in radicals R2, R3, R4, R8, R11, R12 and R' is a radical derived from a mono- or poly-cyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

any "C1-C6 alkyl", "C2-C7 alkanoyl" and C2-C6 alkenyl in radicals R2, R3, R4, R8 R11, R12 and R' may be substituted by at least one group selected from halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C6-C14 aryl, and heteroaryl;

any "C6-C14 aryl", "C7-C15 aroyl" and "heteroaryl" in radicals R2, R3, R4, R8 R11, R12 and R' may be substituted by at least one group selected from halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, and C5-C6 cycloalkyl;

and pharmaceutically acceptable salts thereof.

105. A pharmaceutical composition according to Claim 104 comprising a compound of the formula Ia:

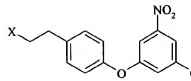


(Ia)

wherein R2, R4 and R8 are as defined in Claim 104.

106. A pharmaceutical composition according to Claim 105, wherein R2 is –OR', R4 is nitro, and R8 is C1-C6 alkyl optionally substituted by at least one group selected from halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C6-C14 aryl, and heteroaryl, wherein R', R11 and R12 are as defined in Claim 104.

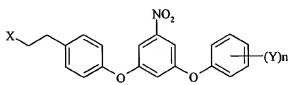
107. A pharmaceutical composition according to Claim 106 comprising a compound of the formula Ib:



(Ib)

wherein X is -COOR' or -CONR11R12, wherein R', R11 and R12 are as defined in Claim 104.

108. A pharmaceutical composition according to Claim 107 comprising a compound of the formula Ic:



(Ic)

wherein Y is halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, -SO_3H, -$SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl, n is an integer from 0 to 5, X is as defined in Claim 4, and R', R11 and R12 are as defined in Claim 104.

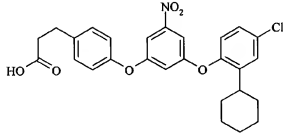
109. A pharmaceutical composition according to Claim 108, wherein n is 2, one Y is halogen and another Y is C5-C6 cycloalkyl, and X is as defined in Claim 107.

110. A pharmaceutical composition according to Claim 109 comprising a compound of the formula Id:

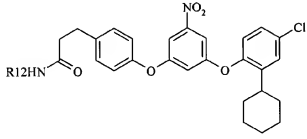
(Id)

wherein X is -COOR' or –CONR11R12, wherein R' and R11 are hydrogen and R12 is C1-C6 alkyl optionally substituted by halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C6-C14 aryl, or heteroaryl.

111. A pharmaceutical composition according to Claim 110 comprising the compound herein designated **Compound 1** of the formula:



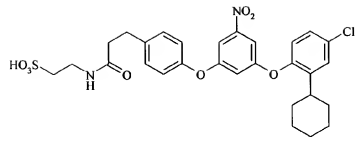
112. A pharmaceutical composition according to Claim 110 comprising a compound of the formula Ie:



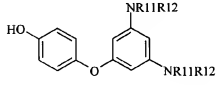
(Ie)

wherein R12 is C1-C6 alkyl optionally substituted by halogen, -OR', -SR', -COOR', nitro, $-SO_3H$, C6-C14 aryl, or heteroaryl, wherein R' is as defined in Claim 104.

113. A pharmaceutical composition according to Claim 112 comprising the compound herein designated **Compound 2** of the formula:



114. A pharmaceutical composition according to Claim 105 comprising a compound of the formula If:



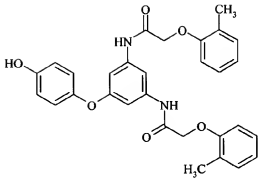
(If)

wherein R11 and R12 are as defined in Claim 104.

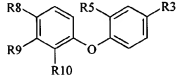
115. A pharmaceutical composition according to Claim 114, wherein R11 is hydrogen and R12 is C2-C7 alkanoyl optionally substituted by halogen, -OR', -SR', -COOR', nitro, $-SO_3H$, C6-C14 aryl, and heteroaryl; or C7-C15 aroyl optionally substituted by at least one group selected from halogen, -OR', -SR', -COOR', nitro, $-SO_3H$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, and C5-C6 cycloalkyl, wherein R' is as defined in Claim 104.

116. A pharmaceutical composition according to Claim 115, wherein R12 is C2-C7 alkanoyl optionally substituted by –OR', wherein R' is a C6-C14 aryl substituted by C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl.

117. A pharmaceutical composition according to Claim 116 comprising the compound herein designated **Compound 3** of the formula:



118. A pharmaceutical composition according to Claim 104 comprising a compound of the formula Ig:

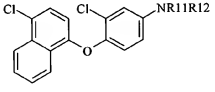


(Ig)

wherein R3, R5, R8, R9 and R10 are as defined in Claim 104.

119. A pharmaceutical composition according to Claim 118, wherein R3 is -NR11R12, R5 and R8 are halogen, R9 and R10 together with the carbon atoms to which they are attached form a condensed benzene ring, and wherein R11 and R12 are as defined in Claim 104.

120. A pharmaceutical composition according to Claim 119 comprising a compound of the formula Ih:

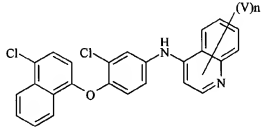


(Ih)

wherein R11 and R12 are as defined in Claim 104.

121. A pharmaceutical composition according to Claim 120, wherein R11 is hydrogen and R12 is C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or heteroaryl optionally substituted by halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl, wherein R' is as defined in Claim 104.

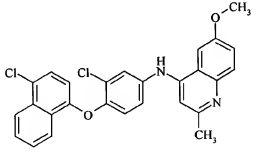
122. A pharmaceutical composition according to Claim 121 comprising a compound of the formula Ii:



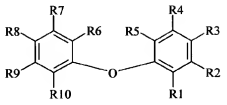
(Ii)

wherein V is halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl, n is an integer from 0 to 6, and R', R11 and R12 are as defined in Claim 104.

123. A pharmaceutical composition according to Claim 122 comprising the compound herein designated **Compound 4** of the formula:



124. Use of a diphenyl ether of the Formula I:



(I)

wherein

R1, R5, R6 and R7 each independently represents hydrogen or halogen;

R2, R3, R4 and R8 each independently represents hydrogen, halogen, nitro, -OR', -SR', -NR11R12, -COOR', -CONR11R12, $-SO_3H$, $-SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, C6-C14 aryl or heteroaryl;

R9 and R10 each independently is hydrogen or halogen, or R9 and R10 together with the carbon atoms to which they are attached, form a condensed benzene ring;

R11 and R12 each independently represents hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or heteroaryl;

or R11 is H and R12 is C2-C7 alkanoyl or C7-C15 aroyl, or R11 and R12 together with the N atom to which they are attached form a saturated 5-7 membered heterocyclic ring containing one to three heteroatoms selected from N, O and/or S;

R' is hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or heteroaryl;

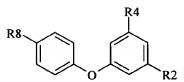
"heteroaryl" in radicals R2, R3, R4, R8, R11, R12 and R' is a radical derived from a mono- or poly-heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

any "C1-C6 alkyl", "C2-C7 alkanoyl" and "C2-C6 alkenyl" in radicals R2, R3, R4, R8 R11, R12 and R' may be substituted by at least one group selected from halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C6-C14 aryl, and heteroaryl;

any "C6-C14" aryl, "C7-C15" aroyl and "heteroaryl" in radicals R2, R3, R4, R8 R11, R12 and R' may be substituted by at least one group selected from halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, and C5-C6 cycloalkyl;

or of a pharmaceutically acceptable salt therof, for the preparation of a pharmaceutical composition .

125. Use according to Claim 124 of a compound of the formula Ia:

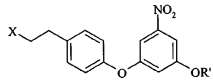


(Ia)

wherein R2, R4 and R8 are as defined in Claim 124.

126. Use according to Claim 125, wherein R2 is –OR', R4 is nitro, and R8 is C1-C6 alkyl optionally substituted by at least one group selected from halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C6-C14 aryl, and heteroaryl, wherein R', R11 and R12 are as defined in Claim 124.

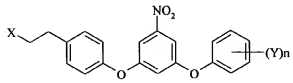
127. Use according to Claim 126 of a compound of the formula Ib:



(Ib)

wherein X is -COOR' or -CONR11R12, wherein R', R11 and R12 are as defined in Claim 124.

128. Use according to Claim 127 of a compound of the formula Ic:



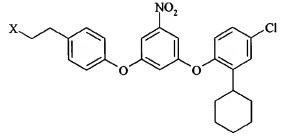
(Ic)

wherein Y is halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl,

n is an integer from 0 to 5, X is as defined in Claim 4, and R', R11 and R12 are as defined in Claim 124.

129. Use according to Claim 128, wherein n is 2, one Y is halogen and another Y is C5-C6 cycloalkyl, and X is as defined in Claim 127.

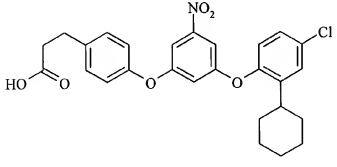
130. Use according to Claim 129 of a compound of the formula Id:



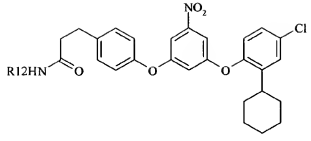
(**Id**)

wherein X is -COOR' or –CONR11R12, wherein R' and R11 are hydrogen and R12 is C1-C6 alkyl optionally substituted by halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C6-C14 aryl, or heteroaryl.

131. Use according to Claim 130 of the compound herein designated **Compound 1** of the formula:



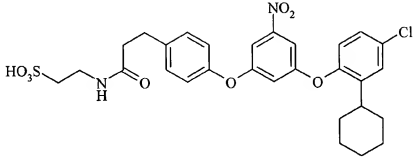
132. Use according to Claim 130 of a compound of the formula Ie:



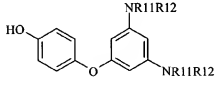
(Ie)

wherein R12 is C1-C6 alkyl optionally substituted by halogen, -OR', -SR', -COOR', nitro, $-SO_3H$, C6-C14 aryl, or heteroaryl, wherein R' is as defined in Claim 124.

133. Use according to Claim 132 of the compound herein designated **Compound 2** of the formula:



134. Use according to Claim 125 of a compound of the formula If:



(If)

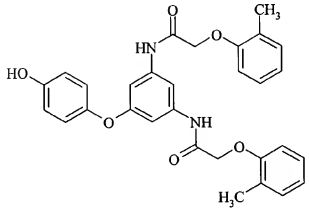
wherein R11 and R12 are as defined in Claim 124.

135. Use according to Claim 134, wherein R11 is hydrogen and R12 is C2-C7 alkanoyl optionally substituted by halogen, -OR', -SR', -COOR', nitro, $-SO_3H$, C6-C14 aryl, and

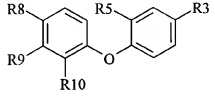
heteroaryl; or C7-C15 aroyl optionally substituted by at least one group selected from halogen, -OR', -SR', -COOR', nitro, -SO_3H, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, and C5-C6 cycloalkyl, and R' is as defined in Claim 124.

136. Use according to Claim 135, wherein R12 is C2-C7 alkanoyl optionally substituted by –OR', wherein R' is a C6-C14 aryl substituted by C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl.

137. Use according to Claim 136 of the compound herein designated **Compound 3** of the formula:



138. Use according to Claim 124 of a compound of the formula Ig:

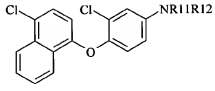


(Ig)

wherein R3, R5, R8, R9 and R10 are as defined in Claim 124.

139. Use according to Claim 138, wherein R3 is -NR11R12, R5 and R8 are halogen, R9 and R10 together with the carbon atoms to which they are attached form a condensed benzene ring, and wherein R11 and R12 are as defined in Claim 124.

140. Use according to Claim 139 of a compound of the formula Ih:

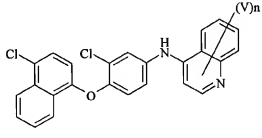


(Ih)

wherein R11 and R12 are as defined in Claim 124.

141. Use according to Claim 140, wherein R11 is hydrogen and R12 is C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or heteroaryl optionally substituted by halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl, wherein R' is as defined in Claim 124.

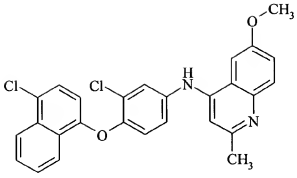
142. Use according to Claim 141 of a compound of the formula Ii:



(Ii)

wherein V is halogen, -OR', -SR', -NR11R12, -COOR', -CONR11R12, nitro, $-SO_3H$, $-SO_2NR11R12$, C1-C6 alkyl, C1-C6 alkoxy, C2-C6 alkenyl, or C5-C6 cycloalkyl, n is an integer from 0 to 6, and R', R11 and R12 are as defined in Claim 124.

143. Use according to Claim 142 of the compound herein designated **Compound 4** of the formula:



1/1

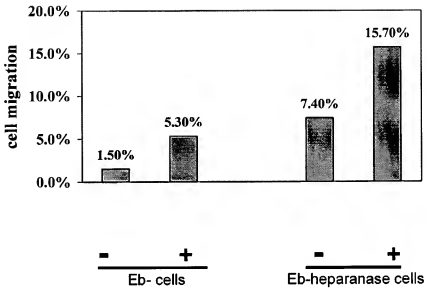


Fig. 1A

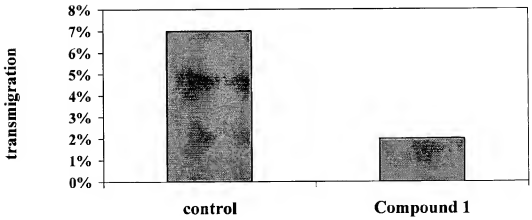


Fig. 1B

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(54) Title: DIPHENYL ETHER DERIVATIVES AND THEIR USES AS HEPARANASE INHIBITORS

(57) Abstract: The invention provides diphenyl ether compounds as heparanase inhibitors suitable for treatment of diseases and disorders caused by or associated with heparanase catalytic activity such as cancer, inflammatory disorders and autoimmune diseases.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
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B. FIELDS SEARCHED
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CAS ONLINE, CAPLUS, BIOSIS, SCISEARCH, USPATFUL, PCTFUL
structure seached and search terms:tumor, non-solid cancers, leukimia, Hodgkin's disease, cell proliferative disorders

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- A	WO9964044 A1 (ADVANCED MEDICINE, INC) 16 December 1999 (16.12.1999), see full text.	1, 124 ----- 2-123, 125-143N
X --- A	Database CAPLUS on STN,N.D. Zelinsky Inst. Org. Chem., (Moscow, Russia), AN:1995:656708, SHEVELEV et al., Phenol substitution of nitro groups in 1,3,5-trinitrobenzene-method of preparation of 5-nitroresorcinol diaryl ethers and 3,5-dinitrophenylaryl ethers, abstract, Izvestiya Akademii Nauk, Seriya Khimicheskay, 1995, Vol. 2, Pages 393-394.	124 ------ 1-123, 125-143

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